

White Matter Integrity and Executive Functioning in Fetal Alcohol Spectrum Disorders

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ABSTRACT

Background: Executive functioning deficits are observed frequently in children with fetal alcohol spectrum disorders. In addition, neuroimaging studies have identified patterns of specific white matter damage in children with a history of prenatal alcohol exposure (PAE). This study investigated whether disruptions to white matter integrity, specifically in the frontal lobes, mediates the executive functioning impairments reported in children with a history of PAE.

Methods: A sample of 54 children born to prospectively recruited mothers in Cape Town, South Africa, underwent neuropsychological testing and neuroimaging. Standard neuropsychological tests assessed performance across four domains of executive functioning (attentional control, information processing, cognitive flexibility, and goal setting). Diffusion tensor imaging (DTI) assessed the integrity of various white matter tracts in the brain.

Results: Children with FASD showed significant impairments on tests of cognitive flexibility, compared to HE children. Significant structural impairments to white matter integrity were also seen in children with FASD compared to Controls. A subset of these were also observed in HE children. However, white matter integrity did not significantly mediate executive function test performance in children with a history of PAE.

Conclusion: This is the first study to investigate mediation of executive dysfunction by damage to white matter integrity in children with a history of PAE. The effects of PAE on executive functioning was not mediated by structural abnormalities in white matter integrity. This study contributes to the literature by increasing knowledge of the profile of executive functioning deficits in children with a history of FASD, taking the position that there are multiple measures of the executive function construct.

Keywords: diffusion tensor imaging; executive functioning; fetal alcohol spectrum disorders; fetal alcohol syndrome; prenatal alcohol exposure; white matter.

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Alcohol consumption during pregnancy is one of the leading causes of preventable developmental disability (Treit et al., 2013). Alcohol is a teratogen that causes a range of physical, cognitive, and behavioral deficits in the developing embryo (Riley, Infante, & Warren, 2011). A growing body of neuroimaging studies has investigated structural anomalies that underlie cognitive deficits frequently observed in fetal alcohol spectrum disorders (FASD; Gautam, Nunez, Narr, Kan, & Sowell, 2014; Wozniak & Muetzel, 2011; Fan et al., in press). Given that executive function deficits are widely reported in individuals with FASD (Kodituwakku & Kodituwakku, 2014; Mattson, Crocker, & Nguyen, 2011), and that recent studies have shown that white matter damage is frequently linked to executive dysfunction following traumatic brain injury (Stuss, 2011a; Voineskos et al., 2012), it is of interest to examine the relation between executive functioning and white matter integrity in FASD.

FASD: Diagnosis and prevalence

The umbrella term *fetal alcohol spectrum disorders* describes a continuum of effects caused by prenatal alcohol exposure (PAE; Hoyme et al., 2005). Variability in presentation of FASD depends on the quantity and timing of PAE, as well as the presence of, and interactions between, environmental and genetic factors (Dodge, Jacobson, & Jacobson, 2014; Jacobson et al., 2006; Treit et al., 2013; Warren & Li, 2005). The FASD spectrum is comprised of four discrete diagnostic categories: fetal alcohol syndrome (FAS), partial FAS (pFAS), alcohol-related birth defects (ARBD), and alcohol-related neurodevelopmental disorder (ARND). Classification into one of those four categories is based on a confirmed history of PAE, as well as assessment of facial dysmorphology, physical growth deficiencies, and cognitive-behavioral impairments. The most severe phenotype is FAS, characterized by craniofacial irregularities (e.g., short palpebral fissures, thin upper lip, and smooth philtrum), growth retardation, and central nervous system (CNS) dysfunction. A confirmed history of PAE is not required when all of these criteria are met and after other genetic disorders that may mimic FAS are ruled out. A diagnosis of pFAS is given when two of the three key characteristic facial anomalies are present, along with either CNS dysfunction, growth retardation, or cognitive-behavioral abnormalities. ARBD is diagnosed when alcohol-related congenital structural deficits are observed in the absence of CNS dysfunction. ARND is diagnosed when both cognitive-behavioral abnormalities and CNS dysfunction are present,

but the child does not meet criteria for either FAS or PFAS (Hoyme et al., 2005; Jacobson et al., 2008).

Globally, the highest rates of FASD are reported in the Western Cape province of South Africa (Riley et al., 2011). A systematic review of worldwide FASD prevalence rates (Roozen, Peters, Townend, Nijhuis, & Curts, 2016) found that South Africa had the highest rates for FAS (55.42 per 1000), followed by Croatia (37.19 per 1000), and Canada (11.73 per 1000). Similarly, South Africa had the highest rates of ARND (20.25 per 1000), followed by the United States (9.07 per 1000). Moreover, South Africa had the third-highest rates of pFAS (28.25 per 1000), following Croatia (43.01 per 1000) and Italy (36.89 per 1000). The high prevalence of FASD in many Western Cape communities can be explained by a culture of heavy recreational alcohol consumption, which can in turn be attributed to poor psychosocial conditions and the legacy of the traditional *dop* system, whereby farm laborers were paid, in part, with wine (Jacobson et al., 2008).

Executive Functioning in FASD

Clinicians and researchers observe a wide range of cognitive-behavioral deficits in children with FASD. These deficits include diminished general intellectual functioning, as well as impairments in discrete cognitive domains, including learning and memory, visual-spatial abilities, number processing, social cognition (e.g., theory of mind), attention, and executive functioning (Lewis et al., 2015; Lindinger et al., 2016; Mattson et al., 2011). Additionally, children with FASD are more vulnerable to secondary psychiatric disabilities (e.g., depression), and are more likely to display emotional lability and unusual physiologic responses, than their typically developing peers (Hoyme et al., 2005; Kodituwakku & Kodituwakku, 2014).

Of particular interest are the executive functioning impairments that are frequently observed in individuals with FASD. The term *executive function* refers to an array of cognitive skills and behaviors, including goal selection, planning, initiation, and cognitive flexibility (Rasmussen et al., 2013). Neuroimaging and lesion studies suggest that these skills and behaviors, which are fundamental to cognitive control, emotional regulation, and social interaction (Anderson, 2002), are mediated, predominantly, by the frontal lobes and associated networks (Cummings, 1993; Stuss, 2011b).

Although contemporary research makes it clear that executive functioning cannot be conceptualized as a single construct, there is no clear consensus on how the cognitive domain should be partitioned (Miyake, Friedman, Emerson, Witzki, & Howerter, 2000; Stuss, 2011b). For the purposes of application to pediatric populations, however, it is useful to adopt

Anderson's (2002) multiprocess model of executive function (see Figure 1). He suggested four distinct, yet inter-related, executive function domains: attentional control, information processing, cognitive flexibility, and goal setting. The four domains differ in their developmental trajectories. For instance, attentional control is observed by 12 months of age, and develops rapidly during infancy and early childhood. Information processing is apparent in infants at 6 months of age (Jacobson, Jacobson, Sokol, Martier, & Ager, 1993). Cognitive flexibility and goal setting begin to emerge at 3 years old, and are relatively mature by 12 years of age (Anderson, 2002). Most executive functions, however, continue to mature and develop through mid-adolescence or early adulthood (Best & Miller, 2010). Given that executive functions rely on the integrity of the frontal lobes, functional improvements in these domains correlate to neurophysiological developments (Stuss, 2011b).

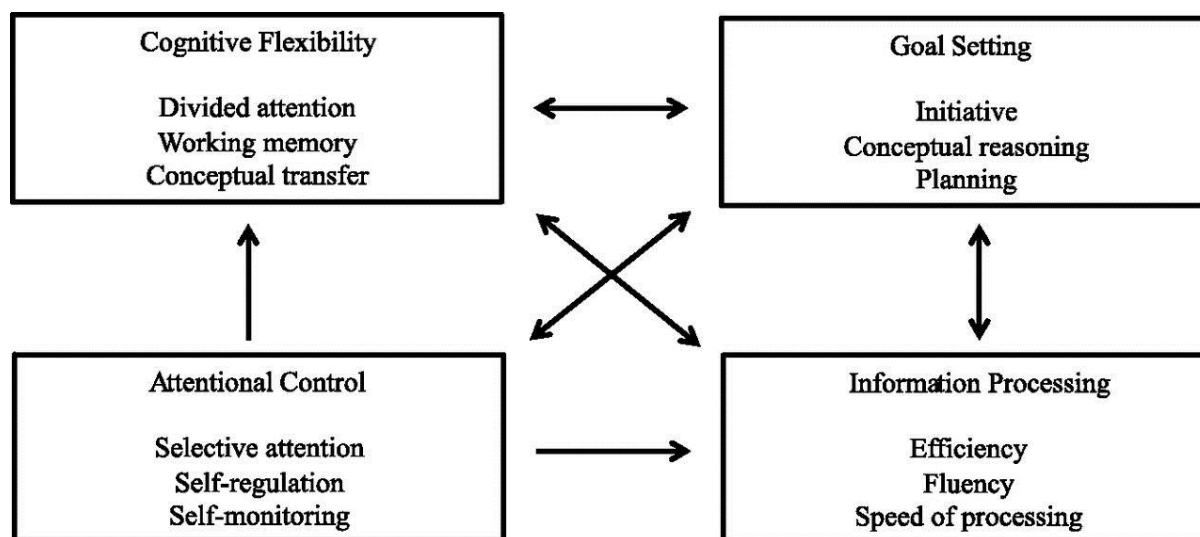


Figure 1. Anderson's (2002) multiprocess model of executive functions.

Executive dysfunction occurs when there is deficient performance on one or more standardized tests of executive function. Neuropsychological studies have shown that children with a history of PAE show impaired performance on tests of set-shifting and working memory (these tests assess performance in Anderson's cognitive flexibility domain), cognitive planning (the goal setting domain), fluency and processing speed (the information processing domain), and response inhibition and self-regulation (the attentional control domain; Kodituwakku & Kodituwakku, 2014; Mattson et al., 2011). For instance, Rasmussen et al. (2013) administered the NEPSY-II standardized neuropsychological battery to 32 children with FASD and 36 healthy control participants, aged between 6 and 16 years. On average, those in the FASD group scored significantly lower than controls on NEPSY-II

subtests measuring set-shifting, concept formation, and inhibition. This pattern of results held even when IQ was statistically controlled, suggesting that overall lower general intellectual functioning does not account completely for executive functioning deficits in FASD.

Neuroimaging studies have found structural abnormalities specific to FASD. These abnormalities are present especially in the cerebellum, corpus callosum, basal ganglia, hippocampus, and frontal lobes (for a review, Nunez, Roussotte, & Sowell, 2011; also see Meintjes et al., 2014). Additionally, PAE negatively affects all stages of brain development (Riley et al., 2011). Of particular relevance here is the stage relating to myelination of axonal tracts and fibers. Uninterrupted development during that stage is essential for continued efficiency of neural transmission, and, consequently, for unimpaired performance on cognitive tests assessing processing speed (Wozniak & Muetzel, 2011).

The white matter that arises from this myelination process appears to be particularly vulnerable to the teratogenic effects of alcohol during development (Jacobson et al., 2011; Riley et al., 2011). A longitudinal neuroimaging study found that although white matter volume increased linearly, with age, in both FASD and unexposed control groups, FASD participants had consistently less white matter than controls (Lebel et al., 2012).

In neuroscience research, white matter integrity is measured using diffusion tensor imaging (DTI). This is a non-invasive magnetic resonance imaging technique that provides an accurate measure of tissue organization and integrity (Wozniak & Muetzel, 2011). More specifically, DTI analyses produce two major indices of white matter integrity: lower fractional anisotropy (FA) values and higher mean diffusivity (MD) values are present in negatively affected white matter compared to normal white matter (Nunez et al., 2011; Treit et al., 2013).

DTI investigations of children with FASD have produced contradictory findings. Although some studies have confirmed the prediction that children with a history of heavy PAE will have significantly lower FA values (i.e., more white matter damage) than controls, specifically within the frontal lobes (Fryer et al., 2009; Lebel et al., 2012), others have reported higher FA and lower MD values (i.e., typical white matter development) in alcohol-exposed participants compared to typically developing controls (Nunez et al., 2011; Treit et al., 2013).

In terms of DTI studies linking structural damage to cognitive-behavioral measures, Spottiswoode et al. (2011) showed that fetal alcohol-related deficits in eye-blink conditioning were mediated by lower FA in the left middle cerebellar peduncles in children who were heavily exposed to alcohol compared to non-exposed controls. The results also showed strong

between-group differences in perpendicular diffusivity, suggesting poorer axon density packing and/or myelination in those with a history of PAE. The study was, however, limited by a small sample (13 children with FASD matched with 12 controls; Spottiswoode et al., 2011).

More recently, Fan et al. (in press) performed whole-brain DTI on 54 children with a mean age of 9.4 years (26 with a FAS/pFAS diagnosis, 13 heavily exposed but non-syndromal (HE), and 15 unexposed controls) from a longitudinal, prospectively recruited cohort, to investigate whether PAE-related deficits in cortical white matter integrity was related to poorer performance on tests of IQ, learning and memory, and eye-blink conditioning. Findings suggested four regions (the inferior longitudinal fasciculus [ILF] bilaterally, splenium, and isthmus) with overlapping FA and MD alterations in children with FAS/pFAS. A subset of those regional alterations were also found in HE children, including the left ILF and the splenium of the corpus callosum. In addition, significant correlations were observed between continuous measures of PAE and mean FA and MD values, suggesting dose-dependent impairments in white matter integrity. Fan et al. (in press) also found mediation of cognitive-behavioral deficits, specifically processing speed and eye-blink conditioning, by white matter impairment.

Relations between EF and White Matter Integrity in FASD

Despite more than four decades of research on FASD, there remains limited understanding of the neural substrates that mediate the cognitive-behavioral deficits often observed in children on the spectrum (Jacobson, Jacobson, Stanton, Meintjies, & Molteno, 2011). For instance, one might hypothesize, based on the literature reviewed above, that compromised white matter integrity, specifically within the frontal regions, may underlie executive dysfunction in FASD. However, few studies have tested this hypothesis.

In the published study that is perhaps most closely related to this one, Gautam et al. (2014) investigated how changes in white matter volume related to changes in executive function in 54 typically developing controls and 49 children with FASD. Participants were recruited from a clinical sample, and were aged between 6 and 17 years. Measurement of white matter volume and executive function, specifically attention and working memory, were taken at two time points approximately 3 years apart. Findings indicated that, despite participants in both the alcohol-exposed and the unexposed control groups showing significant improvements in executive functioning over time, children with FASD consistently performed more poorly than controls. Similarly, the rate of increase in white matter volume with age was similar across groups, but children with FASD had consistently

smaller white matter volumes than controls. The conclusions one might draw from that study are limited, however, because (1) only 5 participants in the FASD group (10% of the group) had a full FAS diagnosis, and so, on average, the group represented children with less severe functional deficits (i.e., those who would be expected to have smaller differences in executive functioning from control participants); (2) the study used MRI to measure white matter volume, and not DTI to measure white matter integrity; and (3) the cohort was retrospectively recruited.

Consequently, there remain numerous unanswered questions in this area of the literature. Examination of a prospectively recruited cohort, that includes both FASD diagnosis and continuous measures of PAE (i.e., that can test possible dose-effect relations); that has a substantial number of children with FAS, pFAS, as well as heavily exposed non-syndromal children; that uses multiple measures of the executive functioning construct; and that is sensitive to theoretical models of executive functioning, has considerable potential to address these issues.

Rationale, Specific Aims, and Hypotheses

In light of the aforementioned alcohol-related cognitive-behavioral and structural deficits in children with FASD, it is evident that white matter integrity may be an important neurodevelopmental component of FASD. More specifically, white matter disruption in the frontal lobes, and in pathways leading to and from the frontal lobes, may impair the optimal performance of complex, high-level cognitive processes, such as those needed to perform optimally on tests of executive functioning. Therefore, it is of clinical relevance to investigate whether white matter integrity mediates executive functioning impairments in children with a history of PAE.

In undertaking such an investigation, the current study tested these hypotheses:

1. Children with a history of PAE, compared to non-exposed controls, will show impaired performance on multiple measures of executive functioning.
2. Children with a history of PAE, compared to non-exposed controls, will have structural impairments in white matter integrity.
3. Executive functioning performance of children with a history of PAE will be mediated by white matter integrity.
4. Executive functioning impairments in children with FASD will not be accounted for fully by overall lower general intellectual functioning, or by potentially confounding clinical and sociodemographic variables (e.g., exposure to other teratogenic agents,

maternal age at delivery, and socioeconomic status (SES); Jacobson & Jacobson, 2005).

Methods

Design and Setting

The current study is nested within an ongoing research program investigating neural effects of PAE on cognitive, affective, and behavioral development (see, e.g., Jacobson et al., 2008; Lewis et al., 2016). The data used in this study were obtained from the 9-year follow-up assessment of participants in a prospective longitudinal cohort.

The main study site, where all cognitive tests were administered, was the Child Development Research Laboratory on the University of Cape Town's Health Science Campus. DTI scans were conducted at the Cape Universities Brain Imaging Centre, which is located at the Tygerberg campus of Stellenbosch University. To avoid experimenter bias, all test administrators were blind to the participant's FASD diagnosis and PAE history.

Participants

The sample consisted of 54 children from the prospective longitudinal cohort study. The children in the cohort were between 8 and 10 years of age ($M = 9.38$; $SD = 0.44$).

Participant recruitment and demographic information. The children in this study were born to women living in Cape Town. The mothers, both those who drank and those who did not drink during pregnancy, were recruited between 1999 and 2002 at the antenatal clinic of a midwife obstetric unit serving a disadvantaged, predominantly Cape Coloured community (Jacobson et al., 2008).

Prospective timeline follow-back interviews assessing levels of prenatal alcohol consumption on a day-to-day basis during a typical 2-week period, both at the time of recruitment and conception, were conducted by a research nurse at a local antenatal clinic. The volume of alcohol consumed per day was recorded and converted into ounces of absolute alcohol per day (oz AA/day) and maternal alcohol consumption was recorded using three measures, oz AA/day across pregnancy; oz AA consumed/occasion; and number of drinking days per week. Mothers were invited to participate in the research if they reported that (a) their average consumption level was equal to or above 1.0 oz AA/day (about 2 standard drinks/day), (b) they reported drinking at least 14 drinks/week, or (c) they engaged in binge drinking (4 standard drinks/occasion). Control participants were matched, on gestational age (± 2 weeks), to each drinking mother. The control mothers were invited to participate if they reported drinking $< .05$ oz AA/day and did not binge drink during pregnancy. Drug use during pregnancy was also reported in terms of number of cigarettes smoked per day across

pregnancy, and drug use across pregnancy was reported (for marijuana and cocaine) in terms of the average number of days/month the drug was used. Two subsequent interviews using the timeline follow-back procedure were administered at mid-pregnancy and at 1 month postpartum. The alcohol use across pregnancy and drug use data obtained from the three interviews was averaged to provide a quantitative summary measure of PAE (oz AA/day) and maternal drug use (see Jacobson, Chiodo, Jacobson, & Sokol, 2002).

Women were excluded from the study if they were younger than 18 years or had diabetes, epilepsy, or cardiac problems requiring treatment. Practicing Muslim women were also excluded because their religion prohibits alcohol consumption; therefore, they would have been disproportionately represented in the control group. Exclusion criteria for infants included major chromosomal anomalies, neural tube defects, multiple births, and seizures.

The children were examined by two expert FASD dysmorphologists (H. E. Hoyme and L. K. Robinson) according to standard diagnostic protocols (Hoyme et al., 2005). There was substantial agreement between the dysmorphologists on the assessment of dysmorphic features, including palpebral fissure length, philtrum and vermilion ratings. FASD diagnosis was determined by consensus at a case conference, and children were assigned to one of three diagnostic groups (FAS, pFAS, or non-syndromal heavily exposed (HE)). Children in the HE group were recruited prospectively based on maternal reports of heavy alcohol consumption during pregnancy, but despite demonstrating significant cognitive and behavioral deficits, they lacked the distinctive alcohol-related facial features. The sample for this study was comprised of 7 children with FAS, 19 children with pFAS, 15 children with HE, and 13 control participants.

Ethical approval of human subjects was obtained, for the larger prospective longitudinal cohort study, from the University of Cape Town's Faculty of Health Science Research Ethics Committees (REC REF:187/2008; see Appendix A), as well as from the Wayne State University's Human Investigation Committee (HIC number: 099504B3F; see Appendix B). Informed consent was obtained from the mothers at recruitment as well as at child assessment visits; assent was obtained from the children at the latter visits (see Appendices C and D, respectively). Children received a small gift and mothers received compensation consistent with the guidelines set by the relevant ethics committees. There were no risks associated with the administration of any of the tests. Participants were informed that they may discontinue testing or leave the study at any point in time, without penalty. Each participant's data were recorded with a code number, ensuring confidentiality

and anonymity. The contact details of the principal investigator were given to participants in case they had any questions or concerns.

Materials

The children who participated in the 9-year follow-up assessment were administered a battery of standardized neuropsychological tests and an assessment of general intellectual functioning. Administration occurred over 2 days to avoid possible fatigue effects. On each testing day, mothers and children were provided with breakfast, a snack, and lunch.

Testing was conducted by two experienced Masters-level graduate research assistants, in Afrikaans or English, depending on the primary language used in the child's home and school. Test instructions were translated by a native Afrikaans-speaking Masters-level child psychologist from the original English for Afrikaans-speaking participants, and then back-translated by a fluent Afrikaans speaker.

Maternal drug and alcohol consumption data. As noted above, a timeline follow-back interview protocol (Sokol, Martier, & Ernhart, 1983) was used at recruitment to obtain maternal alcohol consumption data. Questions pertaining to drug and alcohol use are integrated into the interviewing process in order to obtain reliable measures of maternal cigarette and drug use, as well as a calculation of the alcohol exposure measure (average oz AA/day). The maternal alcohol consumption data provided a continuous measurement of PAE, which enabled the assessment of dose-response relationships between PAE and cognitive outcomes (Jacobson et al., 2008).

Executive functioning tests. Executive functioning was assessed using five neuropsychological tests. Each of these tests measured performance within one of the four domains of Anderson's (2002) multiprocess model of executive functioning.

Attentional control. The interference condition of the *Colour-Word Interference Test* from the *Delis-Kaplan Executive Functioning System* (D-KEFS; Delis, Kaplan, & Kramer, 2001) is based on the traditional Stroop task in that it requires participants to inhibit the learned response of word-reading and to identify the ink color instead. Both the test developers and independent researchers report that this D-KEFS subtest has good reliability ($\alpha = 0.62-0.86$) and validity (Delis et al., 2001; Shunk, Davis, & Dean, 2006).

Information processing. The D-KEFS *Verbal Fluency Test* (Delis et al., 2001) has three conditions: letter fluency, category fluency, and category switching. In the letter fluency and category fluency conditions, participants were required to name as many words as possible that began with designated letters (B, R, and S) or that fell within particular categories (animals and boy/girl names). In the category switching condition, participants

were required to generate as many words as possible while alternating between the categories of fruit/vegetables and clothing. Each trial lasted 60 seconds. This task has good reliability ($\alpha = 0.32-0.90$; Shunk et al., 2006) and validity (Delis et al., 2001).

Cognitive flexibility. The *Children's Colour Trails Test* (CCTT; Llorente, Williams, Satz, & D'Elia, 2003) is divided into two sub-tests: CCTT-1 and CCTT-2. During CCTT-1, participants were required to rapidly connect different colored circles in the correct numerical order. During CCTT-2, participants were required to rapidly connect circles numerically while switching between alternative colors. The developers report moderate test-retest reliability ($r_{tt} = .46-.68$), and good construct and cross-cultural validity.

The *Digit Span Backwards* subtest of the *Wechsler Intelligence Scale for Children-Fourth Edition* (WISC-IV; Wechsler, 2003) also assessed cognitive flexibility, specifically working memory. This subtest required participants to repeat a series of digits, of steadily increasing length, in the opposite order from that in which it was presented by the examiner. This WISC-IV subtest has good reliability and validity (Wechsler, 2003).

Goal setting. In the *Tower of London-Second Edition* (TOL; Culbertson & Zillmer, 2001), the examiner arranged three beads (one red, one blue, one green) into a particular configuration on his/her pegboard. The participant was then required to replicate the configuration, within a time limit of 2 minutes, on a separate pegboard, in as few moves as possible. The TOL is a widely-used, reliable, and valid measure of problem-solving and planning in children (Culbertson & Zillmer, 2001).

General intellectual functioning test. The *WISC-IV* (Wechsler, 2003) was used to estimate general intellectual functioning. Adding this estimated IQ score to the analysis enabled determination of whether any observed deficits on the executive functioning tests were due primarily to PAE, and not due to compromised overall intellectual functioning (Jacobson & Jacobson, 2005).

Procedure

The project secretary scheduled all appointments. The project driver transported participants to and from the various study sites. Each child was tested individually, and mothers were interviewed separately regarding demographic information and the child's school and health history.

Cognitive testing. Standardized administration, data recording, and scoring procedures were followed. Tests were administered in the same order for each participant.

Diffusion tensor imaging. Participants were familiarized with the scanning procedures using a mock scanner (Meintjes et al., 2010). Following this familiarization, data

were acquired using two DTI scans with alternating phase encoding directions (i.e., anterior-posterior and posterior-anterior (AP-PA)) on a 3T Allegra MRI (Siemens, Erlangen, Germany). Each acquisition used the following parameters: 4 reference images with $b=0$ s/mm^2 and 30 diffusion weighted images (DWIs) with $b=1000$ s/mm^2 ; 72 slices; field of view (FOV)= $230 \times 230 \times 13$ s/mm^3 ; slice thickness 1.8mm; 1.8×1.8 s/mm^2 in-plane resolution; TR 10000ms; TE 88ms. A 3D echo planar imaging (EPI) navigated multiecho magnetization prepared rapid gradient echo (MEMPRAGE; van der Kouwe, Benner, Salat, & Fischl, 2008) structural image (resolution $1.3 \times 1.3 \times 1.0$ mm^3 , FOV= $256 \times 256 \times 167$ mm^3 , 128 slices, TR 2530ms, TI 1100ms, TEs 1.53/3.21/4.89/6.57ms, flip angle 7°) was also acquired for each participant.

Pre-processing: Pre-processing included motion correction using FSL-flirt (Smith et al., 2004) and susceptibility correction in Matlab applied to the AP=PA acquisitions (Andersson, Skore, & Ashburner, 2003). The DTI data were initially inspected visually for the presence of dropout slices. Any subjects with dropout slices in any of their DTI acquisitions were excluded from further analyses. After exclusions, for each volume the resultant displacement relative to the first unweighted (B_0) volume was computed using the three translation parameters from mcflirt in FSL. The resultant displacements for all volumes were below 2.0mm for all subjects included in the analyses, maximum resultant displacement did not differ between diagnostic groups. Rotation in any direction were less than 1.3 degrees in all subjects. To compute z -scores, the mean and standard deviations were calculated based on values between the 25th and 75th percentile limits and generated z -score maps for each acquisition. Any data points more than 3 SDs beyond the mean of the z -score map were discarded. The diffusion tensors were estimated, and the relevant maps of DTI scalar parameters (FA and MD) were generated (see Fan et al., in press).

A mean standard space and white matter mask were created for each subject in the following way: the B_0 volumes were co-registered to each subjects own T1w structural image using nonlinear algorithms in FSL. T1w images of controls were co-registered to a single control image and then averaged to create a mean T1w image. Each subject's T1w and DTI parameter images were transformed to this mean T1w space. As a final step, co-registered FA maps of all subjects were averaged, after which individual DTI parameter maps were co-registered to the mean FA image. The cerebrum was extracted using an MNI $1 \times 1 \times 1$ mm^3 template and thresholded at an average FA > 0.2 to ensure that only white matter was included in the analysis (Mori & van Zijl, 2002). For presentation and reporting of cluster

locations, final clusters were mapped to a 1mm³ MNI pediatric standard image (see Fan et al., in press; Fonov et al., 2011).

Data Management and Statistical Analyses

I conducted most of the statistical analyses using the SPSS version 23.0. The data set was checked and cleaned before inferential statistical tests were run. Following convention, α was set at .05 for decisions pertaining to statistical significance. However, due to the public health context of prenatal alcohol exposure research, there is an increased emphasis on missing real effects in the results. Therefore, I am more willing to tolerate Type I than Type II errors (Jacobson & Jacobson, 2005). The continuous measure of PAE, oz AA/day, was log transformed to correct for skewness. All of the assumptions underlying parametric statistical tests were upheld, unless otherwise stated. The level of PAE was categorized by diagnosis. For the purpose of analysis, three alcohol exposure groups were used (FAS and pFAS participants grouped together, HE, and non-exposed controls).

The analysis proceeded across four distinct stages. First, I compiled a comprehensive table of sample characteristics. Using the data from that table, I identified eight potential confounding variables, some related to the child's characteristics (sex, age at testing, and IQ), and others to maternal characteristics (age at delivery, years of education, cigarettes and marijuana use per day across pregnancy, and SES). Second, dose-response relationships were investigated using one-way analyses of variance (ANOVA) to test for statistically significant differences between the three diagnostic groups (FAS/pFAS vs. HE vs. non-exposed controls) and each of the executive functioning outcome variables. Between-group differences on the DTI outcome variables (FA and MD) were also investigated using one-way ANOVAs. Third, mediation by white matter integrity of effects of PAE on executive functioning was assessed using the Sobel (1982) test. Finally, I conducted multiple regression analyses to examine the relation between a continuous measure of PAE and the executive functioning outcome measures after controlling for potential confounding variables.

Results

Sample Characteristics

Table 1 summarizes the demographic characteristics of the children and mothers in the sample, categorized by diagnostic group. The 54 children were aged between 8.74 and 10.52 years ($M = 9.38 \pm 8.9$). Analyses detected no statistically significant between-group difference with regard to age or sex distribution. Children in FAS/pFAS group did, however, have significantly lower IQ scores.

Regarding maternal and family characteristics, the analysis detected no between-group differences with regard to smoking during pregnancy or to mother's age at delivery. Furthermore, only one mother (in the FAS/pFAS group) used cocaine during pregnancy, and only three mothers (also in the FAS/pFAS group) used marijuana during pregnancy. Finally, mothers in the FAS/pFAS group had completed significantly fewer years of education, and emerged from significantly lower SES backgrounds, than did participants in the other two groups.

Between-Group Comparisons: FAS/pFAS versus HE versus control

Executive functioning tests. A series of one-way ANOVAs investigated between-group differences on the executive functioning outcome measures (see Table 2).

Regarding the tasks assessing *cognitive flexibility*, the analyses detected a significant between-group difference in performance on the Digit-Span Backwards task. A series of Games-Howell post-hoc tests suggested that HE participants performed significantly better on this test than those in the FAS/pFAS group ($p = .017$), but that there were no other significant pairwise differences.

Regarding tasks assessing *goal setting*, those assessing *attentional control*, and those assessing *information processing*, the analyses detected no significant between-group differences.

Overall, then, these results do not confirm the hypothesis that, when divided into the appropriate diagnostic groups, children with a history of PAE are significantly impaired, relative to non-exposed controls, on measures of executive functioning.

DTI outcome measures. Following the standard pre-processing of the DTI data, I conducted a series of one-way ANOVAs to examine the between-group differences with regard to regional FA and MD values (see Table 3).

Regarding *fractional anisotropy* values, the analyses detected statistically significant between-group differences across all measured regions (left ILF, right ILF, right splenium of the corpus callosum, right body of the corpus callosum). A series of Games-Howell post-hoc tests suggested that, for all regions, values were significantly lower in FAS/pFAS participants than in unexposed controls, $ps < .007$. In the right ILF and right body of the corpus callosum, FA values were significantly lower in HE participants than in unexposed controls, $ps < .034$.

Regarding *mean diffusivity* values, the analyses detected statistically significant between-group differences across all measured regions (left ILF, right ILF, right splenium of the corpus callosum 1 and 2, right body of the corpus callosum, left SLF (superior longitudinal fasciculus), left anterior thalamic radiation, right anterior thalamic radiation). A

series of Games-Howell post-hoc tests suggested that, for all regions, values were significantly higher in FAS/pFAS participants than in unexposed controls, $ps < .037$. Additionally, the post-hoc tests suggested that, in some of those regions (viz., the left ILF, the right splenium of the corpus callosum 2, the right body of the corpus callosum, the left anterior thalamic radiation, and the right anterior thalamic radiation), MD values were significantly higher in HE participants than in unexposed controls, $ps < .021$.

This pattern of results confirms the DTI-related hypothesis in suggesting that children with a history of PAE (and especially those who can be diagnosed with either FAS or pFAS) have significantly lower FA values and higher MD values (i.e., structural impairments in white matter integrity) relative to non-exposed controls.

Hierarchical Regression Analyses

Mediation by white matter integrity of the effects of PAE on executive functioning. The continuous measure of PAE (oz AA/day) was significantly related to the Digit Span Backwards standard score, $r = -.294$, $p = .031$, and to the total Verbal Fluency score, $r = -.484$, $p < .001$. PAE was not significantly related to performance on the CCTT, the TOL or the Stroop test. To understand whether lower FA and/or higher MD values mediated the significant association between AA/day and performance on the Digit Span Backwards and Verbal Fluency tests, I created 26 separate regression models, 13 for each of the two EF measures that were significant, reflecting the number of regions of interest. Due to so many analyses being conducted, and to protect against inflated familywise error, the alpha level for these comparisons was set at .004 (i.e., $.05/13$). Tables 4 and 5 summarize results from these mediation analyses.

As Table 4 shows, the effects of PAE on Digit Span Backward performance appeared, at least initially, to be mediated by the following measures of white matter integrity: for FA, the right ILF and the right body of the corpus callosum; and for MD, the right body of the corpus callosum, the left SLF, the left anterior thalamic radiation, and the right anterior thalamic radiation. However, non-significant Sobel tests in each of these cases indicated that these potentially mediating variables did not completely mediate the effect of PAE on cognitive flexibility.

As Table 5 shows, the effects of PAE on Verbal Fluency performance appeared, at least initially, to be mediated by the following measures of white matter integrity: for FA, the ILF bilaterally, the right splenium of the corpus callosum, the right body of the corpus callosum; and for MD, the left and right ILF, the right splenium of the corpus callosum (1 and 2), the right body of the corpus callosum, the left SLF, and the anterior thalamic radiation

bilaterally. However, non-significant Sobel tests in each of these cases indicated that these potentially mediating variables did not completely mediate the effect of PAE on information processing.

Potentially confounding variables in the relationship of PAE to executive functioning. The hierarchical regression models described below examined the degree to which potentially confounding variables accounted for some of the variance in the effects of PAE on executive functioning performance. In each model, PAE was entered first and the potentially confounding variables second, as a block, on a separate step.

The first model examined the influence of three potentially confounding variables (child's IQ, family SES, and primary caregiver's years of education) on the relationship between PAE and performance on the Digit Span Backwards test (i.e., the measure of cognitive flexibility). These potentially confounding variables were chosen because of their significant association with performance on the Digit Span Backwards test, $r = .724, p < .001$, $r = .437, p < .001$, and $r = .425, p = .001$, respectively. Although PAE accounted for a significant proportion of the variance in test performance at Step 1, its effects did not remain significant when the confounding variables were entered into the model (see Table 6). Of the three confounding variables, only IQ was a significant predictor of test performance when entered alongside PAE, $F(4, 49) = 14.880, p < .001$.

Regarding the assumptions underlying the regression model, the average VIF score was not substantially greater than 1 ($M_{VIF} = 1.4$), indicating that there was no multicollinearity between predictors. The Durbin-Watson statistic was 2.43, suggesting independence of model residuals. The plot of standardized residuals against standardized predicted residuals indicated that the assumption of homoscedasticity was upheld. In addition, the assumption of normality of standardized residuals was met, $W(54) = .986, p = .757$. Therefore, this model can safely be generalized beyond this sample (Field, 2009).

Regarding the regression model diagnostics, Cook's distance was within the accepted limits (< 1) given the sample size. Mahalanobi's distance had a maximum value of 16.877, which is slightly above the conventional cut-off of 15 (Field, 2009), and could indicate a possible influential case in the distribution of residual scores. This influential case was identified in the distribution of PAE, but it was not recorded because the distribution of PAE had already been normalized. On this basis, one should exercise caution when generalizing this model beyond this sample (Field, 2009).

The second model examined the influence of one potentially confounding variable (marijuana use across pregnancy) on the relationship between PAE and performance on the

CCTT (i.e., a second measure of cognitive flexibility). This potentially confounding variable was chosen because of its significant association with performance on the CCTT test, $r = .562, p < .001$. PAE did not account for a significant proportion of the variance in test performance at initial entry (see Table 6). However, marijuana use across pregnancy was a significant predictor of test performance when entered alongside PAE, $F(2,51) = 12.334, p < .001$.

Regarding the assumptions underlying the regression model, the average VIF score was not greater than 1 ($M_{VIF} = 1.0$) indicating that there was no multicollinearity between predictors. The Durbin-Watson statistic was 1.88, suggesting independence of model residuals. The plot of standardized residuals against standardized predicted residuals indicated that the assumption of homoscedasticity was upheld. In addition, the assumption of normality of standardized residuals was not met, $W(54) = .863, p < .001$. Therefore, caution should be exercised when generalizing this model beyond this sample (Field, 2009).

Regarding the regression model diagnostics, Cook's distance was within the acceptable limits (< 1) given the sample size. Mahalanobi's distance had a maximum value of 45.23, which is above the conventional cut-off of 15 (Field, 1009). Three outliers (i.e., $>3SDs$ above the mean) were identified in the distribution of marijuana use across pregnancy, and two similar outliers were identified in the distribution of CCTT. On this basis, caution should be exercised when generalizing this model beyond this sample (Field, 2009).

The third model examined the influence of one potentially confounding variable (mother's age at delivery) on the relationship between PAE and performance on the Stroop Color-Word Interference test (i.e., the measure of attentional control). This potentially confounding variable was chosen because of its significant association with performance on the Stroop test, $r = -.356, p = .008$. PAE did not account for a significant proportion of the variance in test performance at initial entry (see Table 6). However, mother's age at delivery was a significant predictor of test performance when entered alongside PAE, $F(2,43) = 3.219, p = .050$.

Regarding the assumptions underlying the regression model, the average VIF score was not substantially greater than 1 ($M_{VIF} = 1.2$) indicating that there was no multicollinearity between predictors. The Durbin-Watson statistic was 2.09, suggesting independence of model residuals. The plot of standardized residuals against standardized predicted residuals indicated that the assumption of homoscedasticity was upheld. However, the assumption of normality of standardized residuals was met, $W(46) = .978, p = .541$. Therefore, this model can safely be generalized beyond this sample (Field, 2009).

Regarding the regression model diagnostics, Cook's distance was within the acceptable limits (< 1) given the sample size. Mahalanobi's distance had a maximum value of 15.07, which is marginally above the conventional cut-off of 15 (Field, 1009). Therefore, this model can be generalized beyond this sample (Field, 2009).

The fourth model examined the influence of three potentially confounding variables (child's IQ, family SES, and primary caregiver's years of education) on the relationship between PAE and performance on the Verbal Fluency test (i.e., the measure of information processing). These potentially confounding variables were chosen because of their significant association with Verbal Fluency performance, $r = .503, p < .001$, $r = .352, p = .007$, and $r = .308, p = .017$, respectively. PAE accounted for a significant proportion of the variance in test performance at initial entry, and its effects remained significant when the confounding variables were entered into the model, indicating an independent effect of PAE on Verbal Fluency performance (see Table 6). Of the three confounding variables, only child's IQ was a significant predictor of test performance when entered alongside PAE, $F(4,43) = 5.408, p = .001$.

Regarding the assumptions underlying the regression model, the average VIF score was substantially greater than 1 ($M_{VIF} = 1.67$) indicating possible multicollinearity between predictors. The Durbin-Watson Statistic was 1.75, suggesting independence of model residuals. The plot of standardized residuals against standardized predicted residuals indicated that the assumption of homoscedasticity was upheld. In addition, the assumption of normality of standardized residuals was met, $W(48) = .972, p = .300$. Therefore, this model could safely be generalized beyond this sample (Field, 2009).

Regarding the regression model diagnostics, Cook's distance was within the acceptable limits (< 1) given the sample size. Mahalanobi's distance had a maximum value of 16.88, which is slightly above the conventional cut-off of 15 (Field, 2009), and indicates a possible influential case in the residual distribution. This influential case was identified in the distribution of PAE, but it was not recorded because the distribution of PAE had already been normalized. On this basis, one should exercise caution when generalizing this model beyond this sample (Field, 2009).

None of the potential confounding variables were significantly related to performance on the TOL test. Therefore, no regression analysis was run to examine the relationship between PAE, goal setting, and potential confounding variables.

Discussion

The main aim of the current research was to investigate whether white matter integrity mediates the effect of PAE on four domains of executive functioning (cognitive flexibility, attentional control, goal setting, and information processing). To accomplish this aim, I tested four specific hypotheses. Below, I first discuss the findings relating to each of the four hypotheses in the context of relevant and recently-published literature. Then, I address the limitations of the study and make recommendations for future research based on the current findings.

Executive Functioning across Diagnostic Groups

Hypothesis 1 stated that children with a history of PAE would show impaired performance on multiple measures of executive functioning when compared to non-exposed, typically-developing, demographically similar control children. A series of one-way ANOVAs examined this hypothesis.

Regarding tests assessing *cognitive flexibility*, the analysis showed that children in the FAS/pFAS group performed significantly more poorly than children in the HE group on the Digit Span Backwards test. However, despite the FAS/pFAS participants performing slightly more poorly than those in the Control group, this difference was not large enough for the analyses to detect a statistically significant between-group difference. Contrary to *a priori* predictions, there were no significant between-group differences in CCTT performance. Similarly, on tests assessing *goal setting*, *attentional control*, and *information processing*, the analysis detected no significant between-group differences in performance on the TOL, Stroop, or Verbal Fluency tests. Overall, then, the current observations disconfirmed the hypothesis that, when divided into the appropriate diagnostic groups, children with a history of PAE are significantly impaired, relative to non-exposed controls, on measures of executive functioning.

These results are inconsistent with those reported in a systematic review by Kodituwakku and Kodituwakku (2014). They reviewed 13 studies that observed significant deficits in executive functioning in children with a history of heavy PAE. In addition, specific impairments in working memory, conceptual set shifting, speed of processing, response inhibition, and verbal and non-verbal fluency, have been observed across the range of FASDs (Fugelstad et al., 2015; Mattson et al., 2011). A possible explanation for the discrepancy between the findings of the current study and those of previous studies is that, in the current study, executive functions were defined as comprising of four different domains, whereas in previous studies executive functioning has been defined as a broad term that represents many

different processes. Using multiple measures of the executive function construct, as opposed to conceptualizing it as a single construct, is likely to have produced more accurate results given that global executive function impairment is rarely reported (Anderson, 2002).

Support for this explanation emerges from the fact that the current findings are consistent with those reported by Rasmussen and Bisanz (2009), who also examined the domain-specific profile of executive function deficits in children, aged between 8 and 16 years, with a FASD diagnosis. They found that children with FASD showed relative strengths in the domains of information processing, attentional control, and goal setting, as assessed by performance on tests of category fluency, the Stroop Colour-Word Interference test, and a tower test. They also reported relative weaknesses on the Wisconsin Card Sorting Test (Heaton, Chelune, Talley, Grant, & Berg, 1993), which is designed to measure problem-solving, concept formation and cognitive flexibility (and hence, within the Anderson taxonomy used here, would be classed as an indicator of cognitive flexibility). However, because Rasmussen and Bisanz did not use a control group, it is unclear whether these executive function domains are impaired in children with FASD compared to typically developing peers. Furthermore, Rasmussen and Bisanz found significant age differences in test performance: Older participants performed significantly more poorly than younger participants on tests of letter fluency, and inhibition/switching. Therefore, deficits in these domains (i.e., information control and attentional control) appear to become more pronounced with age (Best & Miller, 2010; Rasmussen & Bisanz, 2009).

Taken together, these results suggest that examination of executive functioning should be domain-specific, and not based on an overall executive function score, because performance by FASD children varies by domain: In some domains they appear relatively strong (i.e., information processing, attentional control, and goals setting), whereas in others they appear relatively weak (i.e., cognitive flexibility). This is further complicated by possible age-related differences in performance in the different domains. That is to say: Children with FASD might grow into deficits. In other words, younger children with FASD may function relatively normally compared to typically developing children, but their performance may be relatively impaired if measured again at an older age. This is because the typically developing children have followed a normal trajectory of development, and so have improved in their test performance, whereas the children with FASD have either stopped developing their performance (i.e., there is no trajectory of improvement), or their trajectory is delayed, so that their relative improvement over time is less than that of typically developing children. These questions cannot be resolved within the design of a cross-

sectional study, thus highlighting the need for longitudinal research on the development of multiple measures of executive functions in children with a history of PAE compared to typically developing controls.

White Matter Integrity across Diagnostic Groups

Hypothesis 2 stated that children with a history of PAE would have structural impairments in white matter integrity when compared to non-exposed, typically-developing, demographically similar control children. A series of one-way ANOVAs examined this hypothesis.

Regarding *FA*, analyses showed that values in the ILF, bilaterally, and in the right splenium and right body of the corpus callosum, were significantly lower in the FAS/pFAS group than in the Control group. Furthermore, HE participants had significantly lower *FA* values than those in the Control group, in the right ILF, and in the right body of the corpus callosum.

Regarding *MD*, analyses showed that values in the ILF, bilaterally, the left SLF, the right body of the corpus callosum, the right splenium (1 and 2), and in the left and right anterior thalamic radiation, were significantly higher in the FAS/pFAS group than in the Control group. Furthermore, HE participants had significantly higher *MD* values than those in the Control group, in the left ILF, the right splenium (2), the right body of the corpus callosum and in the left and right anterior thalamic radiation.

This pattern of results confirms the hypothesis that children with a history of PAE will have structural impairments in white matter integrity when compared to non-exposed controls. These findings are consistent with those reported in other DTI studies on children with a history of PAE. Fan et al. (in press) found significant white matter alterations in the left and right ILF, and in the splenium, of children with FAS/pFAS. Fryer et al. (2009), comparing children with a history of heavy PAE to typically developing non-exposed controls, found significantly lower *FA* values in the right SLF, and in the body of the corpus callosum, and significantly higher *MD* values in the right inferior frontal lobes. Taken together, these findings suggest that, children with a history of PAE display structural impairments in white matter integrity, specifically within the neural regions that support optimal executive functioning (i.e., the frontal lobes, frontal-subcortical circuitry, and the corpus callosum; Cummings, 1993; Fryer et al., 2009).

Mediation of the Association between PAE and Executive Functioning by White Matter Integrity

Hypothesis 3 stated that executive functioning performance of children with a history of PAE would be mediated by white matter integrity. To my knowledge, this is the first study to examine the possible mediating effects of white matter integrity on executive function impairment in children with a history of PAE. PAE was significantly related to performance within the domains of cognitive flexibility (as assessed by performance on the Digit Span Backwards test) and information processing (as assessed by performance on the Verbal Fluency test), but was not significantly related to performance on the CCTT, the TOL, or the Stroop test (i.e., tests measuring cognitive flexibility, goal setting, and attentional control). Therefore, mediation analyses were conducted to investigate whether lower FA and/or higher MD values mediated the significant association between PAE and performance on the Digit Span Backwards and Verbal Fluency tests.

I created 13 regression models to examine the association between PAE and cognitive flexibility, when controlling for structural impairments in white matter integrity in the regions of interest. Results indicated that none of these structural impairments significantly mediated the effects of PAE on test performance. Similar regression models were created to examine the association between PAE and information processing, when controlling for structural impairments in white matter integrity. These models also found no significant mediation. Overall, then, Hypothesis 3 was disconfirmed.

The absence of such mediating effects could suggest that executive functioning performance is relatively independent of white matter integrity in the particular regions measured here, and that PAE impacts on executive functions via mechanisms other than the white matter in those regions. However, previous studies of children with FASD have reported significant correlations between white matter microstructural abnormalities and cognitive deficits, especially on tests assessing executive functioning (Fan et al., in press; Spottiswoode et al., 2011; Wozniak & Muetzel, 2011).

One explanation for this discrepancy between the current findings and those of previous studies relates to the choice of the Sobel test to assess significance of the mediating effect. Some (e.g., Preacher & Leonardelli, 2001) suggest that the Sobel test is better suited for large samples (>200); therefore, the current sample size ($N = 54$) may have delivered inadequate power in the context of that test. Evidence for this account might emerge from the fact that the current analyses indicated that the effect of PAE on cognitive flexibility did shrink, but not significantly so, upon the addition of some mediator variables (viz., FA values

of the right ILF and the right body of the corpus callosum, and MD values of the right body of the corpus callosum, the left SLF, and the anterior thalamic radiation bilaterally). Furthermore, the effect of PAE on information processing also shrank, but again not significantly so, upon the addition of some mediator variables (viz., FA values in the ILF bilaterally, the right splenium of the corpus callosum, and the right body of the corpus callosum, and the MD values in ILF bilaterally, the splenium (1 and 2), the right body of the corpus callosum, the left SLF, and the anterior thalamic radiation bilaterally). Therefore, using a mediation test with more statistical power may have yielded significant results within this sample.

Relation between PAE and Executive Functioning when Controlling for Potential Confounding Variables

When investigating mechanisms that might underlie cognitive and behavioral impairments in children with FASD, it is important to consider clinical and sociodemographic variables as potential confounders (Jacobson & Jacobson, 2005). Thus, Hypothesis 4 stated that executive functioning impairments in children with a history of PAE would be due primarily to the effects of PAE, and would not be accounted for primarily by the effects of potential confounding variables. A series of hierarchical regression models tested this prediction. Preliminary analyses were used to identify potential confounding variables (i.e., clinical and/or sociodemographic variables that showed at least trend-level ($p < .10$) associations with performance on the various tests of executive functioning).

The first of these models examined the association between PAE and cognitive flexibility (assessed by the Digit Span Backwards test) while controlling for child's IQ, family SES, and primary caregiver's years of education. The second examined the association between PAE and cognitive flexibility (assessed by the CCTT) while controlling for marijuana use across pregnancy. The third model examined the association between PAE and attentional control (assessed by the Stroop test) while controlling for mother's age at delivery. Results indicated that, after the potential confounding variables were entered into the model, PAE did not remain a significant predictor of cognitive flexibility or attentional control.

The fourth of these models examined the association between PAE and information processing (assessed by the Verbal Fluency test), when controlling for child's IQ, family SES, and primary caregiver's years of education. Results indicated that, even after these three potential confounding variables were entered into the model, PAE remained a significant predictor of Verbal Fluency performance. Overall, then, these results, partially disconfirm the hypothesis. That is, they suggest that PAE does not have a significant specific effect on the

performance of tests that assess cognitive flexibility, and attentional control, over-and-above the effects of potentially confounding variables. The results do, however, confirm that PAE has a significant specific effect on tests assessing information processing, over-and-above the effects of potentially confounding sociodemographic variables.

The current study used both FASD diagnosis and a continuous measure of PAE (oz AA/day during pregnancy) as a predictor of executive functioning performance. The results indicate that, although no between-group differences were detected in performance on tests of information processing, the continuous measure of PAE was a significant predictor of information processing. This pattern of results is consistent with findings reported by Lindinger et al. (2016). In that study, which examined theory of mind (ToM) ability in children with either FAS/pFAS, HE, and non-exposed control children, performance was assessed, as in this study, using both FASD diagnosis and a continuous measure of PAE (AA/day during pregnancy) as a predictor in regression models. Although no between-group differences were found in performance on ToM tasks, the continuous measure of PAE was a significant predictor of four ToM measures (faux pas comprehension, social cognition, affect recognition, and emotion recognition). Other studies that did not find significant between-group differences, but found significant results when using a continuous measure of PAE include, a study, which examined prospective memory in children with either FAS, pFAS, HE, and non-exposed control children, by Lewis et al. (2016). In that study, findings suggested no between-group differences in performance on a computerized prospective memory task, but the continuous measure of PAE (AA/day during pregnancy) was significantly related to the prospective memory composite score.

Together, these results suggest that ‘executive functioning’ is too broad an umbrella term, and highlights the necessity for considering individual, task-specific, domains when investigating the relationship between PAE and executive functions. These results also suggest that using a continuous measure of PAE is likely to be more sensitive, than using diagnostic groups, in detecting deficits in different domains of executive functioning. Support for this argument comes from evidence that diagnostic groups indicate that exposure occurred, but do not provide a reliable index of the degree of exposure (Jacobson & Jacobson, 2005). Furthermore, there are numerous drawbacks associated with the use of categorical variables, identified in the statistical literature, in clinical research, including a reduction in statistical power to detect relations between variables, underestimation of between-group variations, and increased risk of finding a false positive (i.e., a Type II error;

Altman & Royston, 2006). Therefore, the use of a continuous measure of alcohol exposure is predictive of the quantity of alcohol consumed, and increases the reliability of statistical tests.

Limitations and Recommendations for Future Research

The current research may have been limited by a relatively small sample size. Furthermore, the diagnostic group sizes (FAS/pFAS, HE, and Controls) were unequal. However, these limitations are difficult to remedy due to the non-random recruitment associated with this type of research. Future research should aim to increase the sample size, particularly of the HE and Control groups. One possible remedy future research could undertake to do is to over-recruit participants across all levels of PAE (FAS, pFAS, HE and Controls).

Despite the current study being a part of a larger longitudinal study, it is limited by narrow age ranges of participants. Therefore, it is difficult to extract information regarding the developmental trajectory of executive functions in children with a history of PAE. Future research should focus on longitudinal designs that explore the developmental differences in executive functions for children across the FASD spectrum. Such an approach will enable researchers to investigate the possibility of an altered developmental trajectory of executive functioning in children with a history of PAE. Furthermore, changes in the neural correlates of behavior can then be understood in terms of structural development in the brain (Best & Miller, 2010).

Finally, given that all participants in the current study were recruited from a Cape Coloured community in South Africa, these findings also warrant replication in other populations.

Conclusion

This study is the first to investigate whether white matter integrity mediates the effect of PAE on multiple measures of executive functioning. The findings suggest that, although children with FASD appear impaired relative to typically developing controls on tests of cognitive flexibility, this pattern of results did not persist when potential confounding variables were controlled statistically. In addition, children with a history of PAE show significant structural abnormalities in white matter integrity, specifically within the frontal regions. However, although upon initial inspection it appeared that these structural abnormalities might mediate the relationship between PAE and executive functions, significance testing showed there was no mediating effect. Similarly, an analysis of the relationship between the continuous measure of PAE and the executive function domains suggested that PAE did not significantly predict performance on tests of cognitive flexibility

or attentional control when controlling for potential confounding variables. However, PAE did significantly predict performance on tests of information processing (Verbal Fluency), even after potential confounding variables were controlled statistically. Therefore, this study contributes to the literature by increasing understanding and defining the profile of executive functioning deficits in children with a history of PAE, taking the position that there are multiple measures of the executive function construct.

Table 1
Sample Characteristics (N = 54)

Variable	Group			F / χ^2	p	ESE
	FAS / pFAS ($n = 26$)	HE ($n = 15$)	Control ($n = 13$)			
Child						
Sex (n , % males)	13 (50)	8 (53.33)	7 (53.84)	0.070	1.000	.036
Age at testing (years)	9.28 (0.34)	9.56 (0.55)	9.36 (0.44)	1.733	.185	.074
WISC-IV FSIQ score	64.32 (9.66)	73.12 (7.97)	74.85 (8.97)	7.699	.001**	.232
Mother						
Cigarette use across pregnancy (per day)	8.22 (5.73)	8.13 (7.06)	4.19 (10.91)	1.364	.265	.051
Marijuana use across pregnancy (days/month)	0.18 (0.64)	0 (0)	0 (0)	1.151	.324	.043
Cocaine use across pregnancy (days/month)	0.10 (0.50)	0 (0)	0 (0)	0.529	.592	.020
Age at delivery (years)	28.84 (7.46)	25.17 (4.97)	26.03 (3.28)	2.721	.076	.070
Socioeconomic status (Hollingshead scale)	15.25 (7.05)	24.33 (9.43)	25.0 (6.61)	10.098	< .001***	.284
Primary caregiver's education (years)	7.54 (2.69)	9.47 (2.17)	10.31 (1.44)	7.285	.002**	.222

Note. Means are presented, with standard deviations in parentheses. WISC-IV = Wechsler Intelligence Scale-Fourth Edition; FSIQ = Full Scale IQ. Test statistics were either F or χ^2 depending on whether the variable was categorical or continuous. ESE = effect size estimate (in this case, η^2 for one-way ANOVAs and Cramer's V for chi-squared).

* $p < .05$. ** $p < .01$. *** $p < .001$.

Table 2

Performance on Tests of Executive Functioning: Descriptive statistics and between-group comparisons (N = 54)

Variable	Group			<i>F</i>	<i>p</i>	η^2
	FAS / pFAS (<i>n</i> = 26)	HE (<i>n</i> = 15)	Control (<i>n</i> = 13)			
Cognitive Flexibility						
Digit Span Backwards	4.12 (2.16)	5.60 (1.12)	5.46 (1.51)	4.316	.019*	.145
Children's Color Trails Test	0.85 (0.84)	0.66 (0.51)	1.12 (1.04)	1.069	.351	.040
Goal Setting						
Tower of London	2.35 (1.20)	2.73 (1.03)	2.69 (1.03)	0.737	.483	.028
Attentional Control						
Stroop Interference	98.48 (15.29)	98.13 (15.38)	99.84 (12.83)	0.045	.956	.002
Information Processing						
Verbal Fluency ^a	12.55 (2.77)	14.51 (2.41)	14.74 (4.37)	2.33	.117	.101

Note: Means are presented with standard deviations in parenthesis. **p* < .05. ***p* < .01. ****p* < .001.

^aLevene's test of homogeneity of variance was significant, and therefore the Brown-Forsythe correction was used (adjusted *df* = 2, 26.95)

Table 3

Diffusion Tensor Imaging Measures of White Matter Integrity: Descriptive statistics and between-group comparisons (N = 54)

Variable	Group			<i>F</i>	<i>p</i>	η^2
	FAS / pFAS (<i>n</i> = 26)	HE (<i>n</i> = 15)	Control (<i>n</i> = 13)			
Fractional anisotropy						
L inferior longitudinal fasciculus	0.30 (0.04)	0.32 (0.04)	0.35 (0.04)	6.374	.003**	.200
R inferior longitudinal fasciculus	0.30 (0.04)	0.30 (0.03)	0.35 (0.04)	8.208	.001**	.243
R splenium of corpus callosum	0.40 (0.05)	0.42 (0.08)	0.46 (0.05)	4.827	.015*	.175
R body of corpus callosum	0.33 (0.06)	0.35 (0.04)	0.40 (0.04)	7.410	.001**	.225
Mean diffusivity						
^a L inferior longitudinal fasciculus	0.0008 (0.00004)	0.0008 (0.00002)	0.0008 (0.00004)	10.570	< .001***	.271
R inferior longitudinal fasciculus	0.0008 (0.00003)	0.0007 (0.00004)	0.0007 (0.00003)	7.803	.001**	.234
^b R splenium of corpus callosum 1	0.0008 (0.00006)	0.0008 (0.00009)	0.0008 (0.00004)	4.252	.023*	.140
R splenium of corpus callosum 2	0.0008 (0.00004)	0.0008 (0.00005)	0.0008 (0.00004)	7.094	.002**	.218
R body of corpus callosum	0.0009 (0.00006)	0.0008 (0.00006)	0.0008 (0.00004)	6.592	.003**	.205
L superior longitudinal fasciculus	0.0008 (0.00002)	0.0007 (0.00003)	0.0007 (0.00003)	6.878	.002**	.212
L anterior thalamic radiation	0.0007 (0.00002)	0.0007 (0.00002)	0.0007 (0.00002)	7.347	.002**	.224
R anterior thalamic radiation	0.0008 (0.00002)	0.0008 (0.00004)	0.0007 (0.00003)	7.610	.001**	.230

Note: Means are presented with standard deviations in parentheses. L = left; R = right.

p* < .05. *p* < .01. ****p* < .001.

^aLevene's test of homogeneity of variance was significant, and therefore the Brown-Forsythe correction was used (adjusted *df* = 2.38, .152)

^bLevene's test of homogeneity of variance was significant, and therefore the Brown-Forsythe correction was used (adjusted *df* = 2.30, .598)

Table 4

Mediation Model: Mediation of effect of PAE on cognitive flexibility by white matter integrity (N = 54)

Mediator	PAE		Mediator Variable		Sobel z
	r_1	B_1	r_2	B_2	
Fractional anisotropy					
L inferior longitudinal fasciculus	-0.294*	-0.202	0.324*	0.250	-1.513
R inferior longitudinal fasciculus	-0.294*	-0.494*	0.082	-0.028	0.066
R splenium of corpus callosum	-0.294*	-0.269	0.167	0.009	-0.474
R body of corpus callosum	-0.294*	-0.341*	0.038	-0.109	0.721
Mean diffusivity					
L inferior longitudinal fasciculus	-0.294*	-0.249	-0.224	-0.147	-0.999
R inferior longitudinal fasciculus	-0.294*	-0.287	-0.139	-0.016	-0.105
R splenium of corpus callosum 1	-0.294*	-0.291	-0.152	-0.007	-0.042
R splenium of corpus callosum 2	-0.294*	-0.268	-0.169	-0.071	-0.493
R body of corpus callosum	-0.294*	-0.316*	-0.101	0.048	0.312
L superior longitudinal fasciculus	-0.294*	-0.289*	-0.109	-0.018	-0.124
L anterior thalamic radiation	-0.294*	-0.306*	-0.036	0.045	0.318
R anterior thalamic radiation	-0.294*	-0.336*	0.023	0.131	0.892

Note. Each row summarizes results from a multiple regression analysis examining the effect of PAE and the indicated mediator variable on Digit Span Backwards performance. r_1 indicates the unadjusted correlation between PAE and test performance; B_1 indicates the standardized beta value for PAE when the mediator variable is entered into the regression model; r_2 indicates the unadjusted correlation between the mediator variable and test performance; B_2 indicates the standardized beta value for the mediator variable when PAE is entered into the regression model.

PAE = prenatal alcohol exposure; WISC-IV = Wechsler Intelligence Scale for Children-Fourth Edition; R = right; L = left.

* $p < .05$. ** $p < .01$. *** $p < .001$.

Table 5

Mediation Model: Mediation of effect of PAE on information processing by white matter integrity (N = 48)

Mediator	PAE		Mediator Variable		Sobel z
	r_1	B_1	r_2	B_2	
Fractional anisotropy					
L inferior longitudinal fasciculus	-0.484*	-0.466**	0.220	0.049	-0.350
R inferior longitudinal fasciculus	-0.484*	-0.494**	0.153	-0.028	0.200
R splenium of corpus callosum	-0.484*	-0.479**	0.187	0.012	-0.086
R body of corpus callosum	-0.484*	-0.527**	0.126	-0.109	0.721
Mean diffusivity					
L inferior longitudinal fasciculus	-0.484*	-0.408**	-0.371**	-0.246	-1.582
R inferior longitudinal fasciculus	-0.484*	-0.483**	-0.209	-0.002	-0.014
R splenium of corpus callosum 1	-0.484*	-0.525**	-0.181	-0.082	0.539
R splenium of corpus callosum 2	-0.484*	-0.525***	-0.079	0.133	0.0004
R body of corpus callosum	-0.484*	-0.538**	-0.137	0.115	0.759
L superior longitudinal fasciculus	-0.484*	-0.503**	-0.100	0.059	0.427
L anterior thalamic radiation	-0.484*	-0.508***	-0.045	0.090	0.603
R anterior thalamic radiation	-0.484*	-0.535***	-0.011	0.161	1.102

Note: Each row summarizes results from a multiple regression analysis examining the effect of PAE and the indicated mediator variable on Verbal Fluency performance. r_1 indicates the unadjusted correlation between PAE and test performance; B_1 indicates the standardized beta value for PAE when the mediator variable is entered into the regression model; r_2 indicates the unadjusted correlation between the mediator variable and test performance; B_2 indicates the standardized beta value for the mediator variable when PAE is entered into the regression model. PAE = prenatal alcohol exposure; WISC-IV = Wechsler Intelligence Scale for Children-Fourth Edition; FA = fractional anisotropy; MD = mean diffusivity; R = right; L = left.

* $p < .05$. ** $p < .01$. *** $p < .001$.

Table 6
Hierarchical Regression Analyses Controlling for Potential Confounding Variables

Variable entered	<i>B</i>	<i>SE B</i>	β	<i>p</i>
^aPAE and Cognitive Flexibility Controlling for Child's IQ, family SES and Primary Caregiver's Years of Education (N = 54)				
Step 1				
Constant	5.384	0.345		< .001***
AA/day	-1.239	0.559	-0.294*	.031*
Step 2				
Constant	-5.499	1.571		.001**
AA/day	0.742	0.553	0.176	.186
WISC-IV IQ	0.133	0.023	0.715***	< .001***
SES	0.040	0.029	0.187	.176
Primary Caregiver's Education (years)	0.002	0.098	0.002	.987
^bPAE and Cognitive Flexibility Controlling for Marijuana Use Across Pregnancy (N = 54)				
Step 1				
Constant	0.893	0.157		< .001***
AA/day	-0.074	0.254	-0.041	.771
Step 2				
Constant	0.846	0.131		< .001***
AA/day	-0.183	0.212	-0.100	.392
Marijuana Use Across Pregnancy	1.051	0.212	0.573	< .001***
^cPAE and Attentional Control Controlling for Mother's Age at Delivery (N = 46)				
Step 1				
Constant	99.758	2.996		< .001***
AA/day	-2.463	4.855	-0.076	.614
Step 2				
Constant	121.994	9.404		< .001***
AA/day	2.151	4.956	0.067	.666
Mother's Age at Delivery	-0.892	0.360	-0.381*	.017**
^dPAE and Information Processing Controlling for Child's IQ, family SES, and Primary Caregiver's Years of Education (N = 48)				
Step 1				
Constant	15.206	0.587		< .001***
AA/day	-3.560	0.952	-0.484	< .001***
Step 2				
Constant	7.202	3.561		.049*
AA/day	-2.631	1.255	-0.357	.042*
WISC-IV IQ	0.129	0.052	0.396	.016*
SES	-0.001	0.065	-0.003	0.986
Primary Caregiver's Education (years)	-0.150	0.225	-0.116	.506

Note. Each row summarizes results of a multiple regression analysis examining the effect of confounding variables on the relationship between PAE and performance on tests of executive functioning. *B* indicates the unstandardized beta value, *SE B* indicates the standard error of the unstandardized beta value, and β indicates the standardized beta value. PAE = prenatal alcohol exposure; AA= ounces of absolute alcohol; WISC-IV = Wechsler Intelligence Scale for Children-Fourth Edition; SES = socioeconomic status. ^a $R^2 = .09$ for Step 1, $\Delta R^2 = .46$ for overall model ($p < .001$); ^b $R^2 = .002$ for Step 1, $\Delta R^2 = .32$ for overall model ($p < .001$); ^c $R^2 = .01$ for Step 1, $\Delta R^2 = .124$ for overall model ($p = .05$); ^d $R^2 = .23$ for Step 1, $\Delta R^2 = .10$ for overall model ($p = .001$). * $p < .05$. ** $p < .01$. *** $p < .001$

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Appendix A

Ethics Approval Certificate: University of Cape Town

 UNIVERSITY OF CAPE TOWN <small>UNIVESITHI YASEKAPA - UNIVERSITEIT VAN KAAPSTAD</small>	HUMAN RESEARCH ETHICS COMMITTEE 05 OCT 2012	FACULTY OF HEALTH SCIENCES Human Research Ethics Committee
	FHS016: Annual Progress Report / Renewal	

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
<input checked="" type="checkbox"/> Approved	Annual progress report	Approved until/next renewal date	30/5/2013
<input type="checkbox"/> Not approved	See attached comments		
Signature Chairperson of the HREC	<i>A. M.</i>	Date Signed	9/10/12

Principal Investigator to complete the following:

1. Protocol information

Date form submitted	October 3, 2012		
HREC REF Number	187/2008	Current Ethics Approval was granted until	30/05/2012
Protocol title	Neural Bases of Eyeblink Conditioning in FASD		
Protocol number (if applicable)			
Principal Investigator	A/Prof EM Meintjes		
Department / Office Internal Mail Address	Department of Human Biology, Room 5.14 Anatomy Building, Faculty of Health Sciences, Anzio Road, Observatory		

1.1 Does this protocol receive US Federal funding?	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
1.2 Has sponsorship of this study changed? If yes, please attach a revised summary of the budget.	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No

2. List of documentation

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24 JUL 2013
FHS016: Annual Progress Report / Renewal
 HEALTH SCIENCES FACULTY
 UNIVERSITY OF CAPE TOWN

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
<input checked="" type="checkbox"/> Approved	Annual progress report	Approved until/next renewal date	30.5.2014
<input type="checkbox"/> Not approved	See attached comments		
Signature Chairperson of the HREC		pp T. Burgess	Date Signed 26/07/2013

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date form submitted	18 July 2013		
HREC REF Number	187/2008	Current Ethics Approval was granted until	30/5/2013
Protocol title	Neural Bases of Eyeblink Conditioning in FASD		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?		<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	
If yes, could you please provide the HREC Ref's for all sub-studies? <i>Note: A separate FHS016 must be submitted for each sub-study.</i>			
Principal Investigator	A/Prof EM Meintjes		
Department / Office Internal Mail Address	Department of Human Biology, Faculty of Health Sciences, University of Cape Town, Observatory, 7925		

1.1 Does this protocol receive US Federal funding?	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
1.2 Does this study require full committee approval?	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No

Appendix B

Ethics Approval Certificate: Wayne State University

**WAYNE STATE
UNIVERSITY**

IRB Administration Office
87 East Canfield, Second Floor
Detroit, Michigan 48201
Phone: (313) 577-1628
FAX: (313) 993-7122
<http://irb.wayne.edu>

NOTICE OF FULL BOARD AMENDMENT APPROVAL

To: Sandra Jacobson
Psychiatry
Department of Psychiatry and B

From: Dr. Scott Millis or designee
Chairperson, Behavioral Institutional Review Board (B3)

Date: July 18, 2013

RE: IRB #: 026708B3F
Protocol Title: Neural Bases of Eyeblink Conditioning in FASD
Funding Source: Sponsor: NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM
Sponsor: NATIONAL INSTITUTES OF HEALTH
Protocol #: 0802005726

Expiration Date: February 20, 2014

Risk Level / Category: 45 CFR 46.404 - Research not involving greater than minimal risk
Research not involving greater than minimal risk

The above-referenced protocol amendment, as itemized below, was reviewed by the Wayne State University Institutional Review Board (B3) and is **APPROVED** effective immediately.

- Protocol – Change in enrollment criteria includes the addition of children ages 13-14 to complete the 2r phase of the longitudinal study. This change does not affect risks to participants.
- Oral Assent Script – Resubmission of Oral Assent Script for Ages 7-12 (English Version and Afrikaans Version).
- Assent Form (dated 6/4/2013) – Addition of Documentation of Adolescent Assent Form for Ages 13-14 (English Version and Afrikaans Version).
- Consent Form (dated 4/18/2013, Protocol Version #2r) - Parental Permission/Research Informed Consent (English Version and Afrikaans Version) updated to reflect change in age range and telephone number.
- Consent Form (dated 4/18/2013, Protocol Version #2rr Alternate) - Parental Permission/Research Informed Consent (English Version and Afrikaans Version) updated to reflect change in telephone number.



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NOTICE OF EXPEDITED AMENDMENT APPROVAL

To: Sandra Jacobson
Psychiatry
Department of Psychiatry and B

From: Dr. Deborah Ellis or designee _____
Chairperson, Behavioral Institutional Review Board (B3)

Date: June 11, 2014

RE: IRB #: 026708B3F
Protocol Title: Neural Bases of Eyeblink Conditioning in FASD
Funding Source: Sponsor: NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM
Sponsor: NATIONAL INSTITUTES OF HEALTH
Protocol #: 0802005726

Expiration Date: February 19, 2015

Risk Level / Category: 45 CFR 46.404 - Research not involving greater than minimal risk
Research not involving greater than minimal risk

The above-referenced protocol amendment, as itemized below, was reviewed by the Chairperson/designee of the Wayne State University Institutional Review Board (B3) and is APPROVED effective immediately.

- Protocol - Enrollment criteria modified to reflect change in participants to be seen between ages of 8 to 13 years to ages of 8 to 17 years.
- Protocol - Other - Compensation modified to reflect change to Rand/Dollar conversion update. The compensation remains R150 regardless of USD.
- Consent Form - Parental Permission/Research Informed Consent - English and Afrikaans versions (revision dated 5/27/2014) - Consent Form modified to reflect change in enrollment criteria (increased age range to 8-17 years of age) and compensation amount of R150 due to conversion update between Rand and USD.

Appendix C

Informed Consent Form (English and Afrikaans versions)

Parental Permission/Research Informed Consent

Title of Study: White Matter Integrity and Executive Functioning in FASD

We are pleased to invite you and your child _____ to continue to take part in the study that you have been in since you were pregnant and your baby was born. Please read this form and ask us any questions you have before agreeing to be in the study. The people conducting this study are doctors and scientists from the Faculty of Health Sciences of the University of Cape Town School in South Africa and Wayne State University School of Medicine in the United States: Ernesta Meintjes, Ph.D., and Christopher Molteno, M.D., from University of Cape Town, and Sandra W. Jacobson, Ph.D., and Joseph L. Jacobson, Ph.D., from Wayne State University in the United States. It is being paid for by the National Institute on Alcohol Abuse and Alcoholism in the United States and the Department of Science and Technology and the National Research Foundation of South Africa.

Study Purpose: In this study we want to learn whether some aspects of a child's thinking and behavior are different when a mother drinks or and smokes during pregnancy, and whether genes (characteristics that you inherit from your parents) make it more or less likely that the child will show these differences. Other purposes of the study are to see whether your child's abilities when s/he was a baby and 5 years old predict how he or she is doing at 8-17 years of age. To help decide whether or not to agree to take part with your child in this study, a project staff member has talked with you about the risks and benefits of the study. This consent form summarizes the information given to you by the project staff member during this informed consent process.

The study will use new methods for studying the brain called MRI neuroimaging to better understand how drinking alcohol and smoking during pregnancy can affect a child's development. In neuroimaging, the child lies in a scanner that uses magnets to take pictures of the brain. In this part of the study, we will take pictures on the new scanner at Tygerberg Hospital while your child lies still and watches a video and does some simple finger tapping, attention, and memory tasks.

Study Procedures: If you agree to have your child take part in this study, we will bring you and your child to the our laboratory at the University of Cape Town (UCT) for 2-3 visits that will each take about 4 hours and to Tygerberg Hospital for 1-2 visits that should take about 3-4 hours in total.

During the visits to University of Cape Town, your child will do simple tasks involving finger tapping, attention, learning and memory, arithmetic, word meanings, puzzles, circle drawing, and mazes (Wechsler Intelligence Scale for Children; paced/unpaced finger tapping; Circle Drawing task; timing and pitch perception tasks; California Verbal Learning Test). We will test your child's vision.

In one task, your child will put on a special helmet. While your child is watching a video, a puff of air from the helmet will cause him/her to blink while hearing a tone. We will ask your child questions about the video afterwards.

We will weigh and measure your child and take a photograph to look for facial features that often relate to alcohol exposure during pregnancy.

During this visit, we will ask you some questions about your child's behavior and attention (Disruptive Behavior Disorders assessment), daily activities (Child Behavior Checklist), school and health history, and any medications that s/he is taking.

We will ask you to update us about stressful experiences in your daily life during the past year (Life Events Scale), your current drinking, smoking, and drug use, attention problems you may have had as a child (Barkley-Murphy ADHD Scale), and stressful feelings that you experience, including sadness, anxiety, and distress (Beck Depression Inventory; Structured Clinical Interview for DSM-IV).

At the end of the first visit, our research driver and nurse will take you and your child to a nearby clinic, where a technician/nurse will take a 5 cc blood sample (approximately 1 teaspoon) from your child's vein to test for lead and iron deficiency anemia. About 10 cc of blood (about 2 teaspoons) will be obtained from your child and yourself to study genetic differences that you and your child inherited from your family and have been found to be related to differences in alcohol use, depression, attachment, or child attention/behavior and development. These samples will be stored and used for future genetic analyses.

During the first visit to Tygerberg, your child will first practice the finger tapping, and attention and memory tasks s/he will be doing on a computer while lying in the scanner. During the neuroimaging, your child will lie on a padded plastic bed that slides into the scanner. We will ask him/her to lie as still as possible while the pictures are being taken. Taking these pictures of the brain does not hurt and is used every day by many people in the hospital. During the second visit to Tygerberg, our assistant will again practice the finger tapping and attention/memory tasks with your child and review with him/her the airpuff learning task that s/he has done in our laboratory at UCT. Your child will be shown special goggles that s/he will wear in the scanner and told that s/he will feel the airpuff and hear some tones while watching a video and that we will be asking him/her some questions about the video at the end of the scan. During some of the time in the scanner, your child will watch videos and during some of the time s/he will do the finger tapping and other tasks that were practiced before entering the scanner. There will be two sessions in the scanner at each visit to Tygerberg—both on the same day—one in the morning and one after lunch, which we will give you and your child while you are at Tygerberg. Each session in the scanner will last no longer than 45-60 minutes. Children with the following may not have an MRI but will take part in the rest of the visits: implanted medical devices, such as aneurysm clips in the brain, heart pacemakers, and cochlear (inner ear) implants; lead-based tattoos; or pieces of metal close to or in an important organ (such as, the eye); claustrophobia or fear of being in a small space.

Benefits: There may be no direct benefits for you; however, information from this study may help other people now or in the future. We will give you information about your child's development at this age. We will use the findings from this study for research purposes only. However, if a serious problem is found, we will tell you and refer your child to a doctor and/or someone who can help, if you would like us to do so. If your child is suffering from any major illness, we will send you to Red Cross Children's Hospital. No information about your child will be given to any doctors, hospitals, or schools unless you ask us and allow us to do so in writing.

Risks: None of the procedures we use at UCT or Tygerberg are dangerous for you or your child. The risks of drawing blood include some temporary discomfort or swelling, and rarely, infection. These risks that will be minimized because the procedure will be done by a trained phlebotomist (nurse/technician who has been specially trained to draw blood). We will begin by introducing you and your child to the research staff and will give you both breakfast each

day before the assessment begins. You will be present in a room nearby during all of your child's assessments and will be present with your child during the physical examination and blood draw. During the MRI neuroimaging assessments, certain metal objects, such as, watches, credit cards, hairpins, and writing pens, may be damaged by the MRI scanner or pulled away from the body by the magnet. For these reasons, we will ask your child to remove these before going into the scanner. When the scanner makes the pictures, the bed may shake, and your child will hear loud banging noises. S/he will be given earplugs or headphones to protect the ears. Also, some people feel nervous in a small closed space, such as when they are in the scanner. Your child will be able to see out of the scanner at all times, and we will not start until s/he tells us that s/he is comfortable. S/he will be able to stop the scanning at any time by squeezing a ball that s/he will hold in one hand and can talk to us using an intercom that is built into the scanner. There are no known harmful long-term effects of the magnetic fields used in this study. There is little risk that anything you tell us will be told to people outside the study and we will do everything we can to keep this information secret, as described below, except that evidence of child abuse or neglect will be reported to the appropriate authorities, as required by law, and may report other illegal activities that are reported to us during the visit.

Research Related Injuries: If you or your child is injured during the study, you will get treatment including first aid, emergency treatment and follow-up care, as needed. No reimbursement, compensation, or free medical care is offered by Wayne State University or the University of Cape Town. If you think that your child has suffered a research related injury, let the investigator know right away.

Study Costs: There will be no cost to you or your child for taking part in this research study, and you and your child will be transported to the laboratory at University of Cape Town and Tygerberg Hospital by our driver.

Compensation: For taking part in this research study, we will give you R180 for each visit and a photo of your child, and we will give your child a small gift. You and your child will also be given breakfast and lunch each time you and your child come to University of Cape Town or Tygerberg Hospital.

Confidentiality: We will keep all information collected about you and your child during the study secret to the extent permitted by law. This information will not be used in any way that can allow anyone else to know what you or your child has told us, except that evidence of child abuse or neglect will be reported to the appropriate authorities, as required by law. You and your child's names will not be in the research records, only your code number. We will not give out any information that names you or your child unless you give us written permission, but your records may be reviewed by the study sponsor, the Human Investigation Committee at Wayne State University, the University of Cape Town Research Ethics Committee, or governmental agencies with appropriate regulatory oversight. The list linking names and code numbers will be stored in locked file cabinets in the research laboratory. Only project staff members who need to contact you by telephone or in person will be allowed to look in these files. Information from this study, including photos may be presented in scientific meetings or journals or for teaching purposes, but your and your child's names will be kept secret.

Voluntary Participation/Withdrawal: Taking part in this study is voluntary. You may decide to have your child take part and later change your mind and quit the study. You and

your child are also free not to answer any questions or to stop any task before it is finished. Withdrawal from the study would not lead to any problems for you or your child. The researcher or the sponsor may also stop your child's taking part in this study without your agreeing to it.

Questions: If you have any questions now or in the future, you may contact Drs. Ernesta Meintjes or Christopher Molteno at 021-406-6291 or Dr. Sandra W. Jacobson at 001-313-993-5454. If you have questions or concerns about you or your child's rights as a research participant, you can contact the Chairs of either the University of Cape Town Research Ethics Committee (021 406-6338) or the Wayne State University Human Investigation Committee (001-313-577-1628).

Consent to Participate in a Research Study: To voluntarily agree to have your child take part in this study, you must sign on the line below. If you decide to take part with your child, you or your child may quit at any time. You are not giving up any of your or your child's legal rights by signing this form. Your signature shows that you have read, or had read to you, this whole consent form, including the risks and benefits, and that we have answered all your questions. We will give you a copy of this consent form to take home.

Signature of Parent or Legally Authorized Guardian

Date

Printed Name of Parent or Authorized Guardian

Time

Oral Assent (children age 7-12 years)

Date

**Signature of Witness (When applicable)

Date

Printed Name of Witness

Time

Signature of Person Obtaining Consent

Date

Printed Name of Person Obtaining Consent

Time

** Use when parent has had consent form read to them (i.e., illiterate, legally blind, translated into foreign language).

Afrikaans Consent Form

Toestemming deur Ouer/Ingeligte Toestemming tot Navorsing

Titel van Studie: Wistof Integriteit en Uitvoerende Funksionering in FASD

Jy en u kind _____ word uitgenooi om deel te neem aan ons navorsingstudie. Lees asseblief hierdie vorm deur en vra vir ons enige vrae wat u het voordat u instem om in die studie te wees. Die mense wat hierdie studie doen is dokters en wetenskaplikes aan die Universiteit van Kaapstad se Fakulteit Gesondheidswetenskappe in Suid-Afrika en Wayne State Universiteit Mediese Skool in die Verenigde State: Ernesta Meintjes, Ph.D., en Christopher Molteno, M.D., van die Universiteit van Kaapstad, en Sandra W. Jacobson, Ph.D., en Joseph L. Jacobson, Ph.D., van Wayne State Universiteit in die Verenigde State. Die studie word geborg deur die Nasionale Instituut oor Alkohol Misbruik en Alkoholisme in die Verenigde State en die Departement van Wetenskap en Tegnologie en die Nasionale Navorsingsraad van Suid-Afrika.

Doel van die Studie: In hierdie studie wil ons leer hoe sommige aspekte van hoe 'n kind dink en optree verskillend is wanneer 'n ma drink en/of rook tydens swangerskap, en of gene (eienskappe wat jy van u ouers erf) dit meer of minder waarskynlik maak dat die kind hierdie verskille sal wys. Bykomende doelwitte van die studie is om te ondersoek die mate waartoe toetse wat gedoen is tydens die babajare en tydens 5-jarige ouderdom die kind se prestasie op 8-14-jarige ouderdom voorspel. Om u te help met u besluit om aan die studie deel te neem of nie, het 'n projek personeel die risiko's en voordele met u bespreek. Hierdie toestemmingsvorm is 'n opsomming van die inligting wat aan u gegee is deur die projek personeel tydens hierdie inligting toestemmingsproses.

Hierdie studie sal nuwe metodes wat MRI neurobeelding genoem word, gebruik om beter te verstaan hoe die drink van alkohol en rook tydens swangerskap 'n kind se ontwikkeling kan affekteer. In neurobeelding lê die kind in 'n skandeerder wat magnete gebruik om prentjies van die brein te neem. In hierdie deel van die studie sal ons prentjies neem met die nuwe skandeerder by Tygerberg Hospitaal terwyl u kind stil lê en na 'n video kyk, en sekere eenvoudige take doen waartydens hy/sy sy/haar vingers moet tik, moet aandag gee, en sekere goed moet onthou.

Studie Prosedures: Indien jy instem om u kind aan hierdie studie te laat deelneem, sal ons u en u kind na ons laboratorium bring by die Universiteit van Kaapstad (UK) vir 2-3 besoeke wat elk ongeveer 4 ure sal duur, en na Tygerberg Hospitaal vir 1 - 2 besoeke wat elk omtrent 3-4 ure in totaal behoort te duur.

- Tydens die besoeke aan die Universiteit van Kaapstad sal u kind eenvoudige take doen waartydens hy/sy sy/haar vingers moet tik, moet aandag gee, dinge probeer onthou, somme doen, betekenis van woorde moet gee, legkaarte doen, doolhowe doen, en sirkels teken (Wechsler Intelligensie Skaal vir Kinders; vingertik taak; Sirkel Teken Taak, tyd en frekwensie persepsie take; Californieë Verbale Leer Toets).
- Ons sal u kind se visie toets / toets hoe goed u kind kan sien.
- In een taak sal u kind 'n spesiale helm opsit. Terwyl u kind na 'n video kyk, sal 'n blase lug uit die helm kom wat sal maak dat u kind sy/haar oog knip terwyl hy/sy 'n geluid hoor.
- Ons sal u kind weeg en meet en 'n foto neem om te kyk vir gesigskenmerke wat dikwels verbandhou met alkohol blootstelling tydens swangerskap.

- Tydens hierdie besoek sal ons u ook 'n paar vrae vra oor u kind se gedrag, vermoë om aandag te gee (Steurende Gedragsteuring Toets), daaglikse aktiwiteite (Kindergedrag Vraelys), skool en gesondheidsgeskiedenis, sowel as enige medikasie wat hy/sy neem.
- Ons sal u vra om ons op hoogte te bring oor stresvolle ervarings in u daaglikse lewe gedurende die afgelope jaar (Lewensgebeurtenis Skaal), u huidige drank- en dwelmgebruik en rookpatrone, probleme wat jy as 'n kind mag gehad het om aandag te gee (Barkley-Murphy AAHV Skaal), en stresvolle gevoelens wat jy ervaar, insluitend hartseer, angs, en bekommernis (Beck Depressie Vraelys, Gestruktureerde Kliniese Onderhoud vir DSM-IV).
- Aan die einde van die eerste besoek sal ons navorsingsbestuurder en verpleegster u en u kind neem na 'n nabye kliniek, waar 'n tegnikus/verpleegster 'n 5cc bloedmonster (ongeveer 1 teelepels) van u kind se aar sal neem om te toets vir lood en ystertekort anemie. Omtrent 10 cc bloed (ongeveer 2 teelepels) sal geneem word van u en u kind om genetiese verskille te bestudeer wat verband hou met verskille in alkohol metabolisme, depressie, gehegtheid, of die kind se aandag en ontwikkeling. Hierdie monsters sal gestoor word en gebruik word vir toekomstige genetiese analises.
- Tydens die eerste besoek aan Tygerberg, sal u kind eers die vingertik- en aandag en geheuetake oefen wat hy/sy op 'n rekenaar sal doen terwyl hy/sy in die skandeerder lê. Gedurende die neurobeelding sal u kind op 'n sagte plastiek bed lê wat in die skandeerder inskuif. Ons sal hom/haar vra om so stil as moontlik te lê terwyl die prentjies geneem word. Die afneem van hierdie prentjies (foto's) van die brein maak nie seer nie en word elke dag deur baie mense in die hospitaal gebruik. Tydens die tweede besoek aan Tygerberg sal ons assistent weer die vingertik- en aandag/geheuetake met u kind oefen en met hom/haar hersien die lugblasie leertaak wat hy/sy in ons laboratorium by UK gedoen het. Tydens die skandeerbeseke sal ons vir u kind spesiale brille wys wat hy/sy sal dra in die skandeerder. Ons sal vir u kind sê dat hy/sy die lugblasie sal voel en 'n soort geluid sal hoor terwyl hy/sy na 'n video kyk en dat ons vir hom/haar 'n paar vrae oor die video sal vra aan die einde van die skandering. Vir 'n gedeelte van die tyd in die skandeerder sal u kind na videos kyk, en vir 'n gedeelte van die tyd sal hy of sy die vingertik en ander take doen wat ons geoefen het voordat hy/sy die skandeerder binnegegaan het. Daar sal gedurende elk van die beseke aan Tygerberg twee sessies in die skandeerder wees – albei op dieselfde dag - een in die oggend en een na middagete. Ons sal vir u en u kind middagete gee terwyl julle by Tygerberg is. Elke sessie in die skandeerder sal niks langer as 45-60 minute duur nie. Kinders met enige van die volgende toestande mag nie 'n MRI onderneem nie: ingeplante mediese toestelle soos aneurisme knippies in die brein, hart pasaangeërs, en binne-oor inplantings; loodgebasseerde tattooërmerke, of stukkie metaal naby aan of binne-in 'n belangrike orgaan (soos die oog); engtevrees of die vrees om binne 'n klein ruimte beperk te wees.

Voordele: Daar mag dalk geen direkte voordele vir u wees nie, maar inligting van hierdie studie mag ander mense help, nou of in die toekoms. Jy sal inligting ontvang oor u kind se huidige ontwikkeling op hierdie ouderdom. Ons sal die bevindings van hierdie studie slegs gebruik vir navorsingsdoeleindes. Indien 'n ernstige probleem egter gevind word, sal ons vir u sê en u kind verwys na 'n dokter en/of iemand wat kan help, indien jy dit wil hê. Indien u kind aan enige ernstige siekte ly, sal ons u na die Rooikruis Kinderhospitaal stuur. Geen inligting oor u kind sal uitgegee word aan enige dokters, hospitale, of skole tensy jy dit skriftelik versoek en toelaat nie.

Risiko's: Geen prosedures wat ons by UK of Tygerberg sal gebruik is gevaarlik vir u of u kind nie. Die risiko's van bloedtrek sluit soms 'n bietjie tydelike ongemak of swelling in, en by uitsondering, infeksie. Hierdie risiko's sal verminder word omdat die prosedure deur 'n opgeleide flebotomis (verpleegster/tegnikus wat spesiaal opgelei is om bloed te trek) gedoen sal word. Ons sal begin deur u en u kind aan die projekpersoneel bekend te stel en sal vir julle albei ontbyt gee elke dag voordat die toetse begin. Terwyl al u kind se toetse gedoen word sal jy in 'n vertrek naby u kind wees en jy sal saam met u kind wees tydens die fisiese ondersoek en wanneer die bloed getrek word. Tydens die MRI neurobeelding mag sekere voorwerpe soos horlosies, kredietkaarte, haarknipies en skryfpenne beskadig word deur die MRI skandeerder of deur die magnet weggetrek word van die liggaam. Om hierdie redes sal ons u kind vra om hierdie voorwerpe af te haal voordat hy/sy die skandeerder binnegaan. Wanneer die skandeerder die prentjies neem, mag die bed skud, en u kind sal harde kappgeluide hoor. Hy/sy sal oorpluisies en oorfone gegee word om sy/haar ore te beskerm. Sommige mense voel ook senuweeagtig in 'n klein beperkte spasie soos wanneer hulle in die skandeerder is. U kind sal te alle tye by die skandeerder kan uitsien, en ons sal nie begin voordat hy/sy nie vir ons sê dat hy/sy gemaklik is nie. Hy/sy sal ook enige tyd kan stop deur 'n bal te druk wat hy/sy in een hand sal vashou en hy/sy sal met ons kan praat deur 'n interkom wat in die skandeerder ingebou is. Sover almal weet is daar geen skadelike langtermyn effekte as gevolg van die magnetiese velde wat in hierdie studie gebruik word nie. Daar is baie min kans dat enigiets wat jy vir ons vertel vir ander mense buite die studie gesê sal word en ons sal alles doen wat ons kan om hierdie inligting geheim te hou behalwe, soos hieronder beskryf, indien daar tekens is van kindermishandeling of –verwaarlosing sal dit egter aan die toepaslike owerhede gerapporteer word, soos deur die wet vereis. Ons mag ook ander onwettige aktiwiteite rapporteer wat aan ons tydens die besoek bekend gemaak word.

Navorsingsverwante Beserings: Indien jy of u kind tydens die studie beseer word sal jy behandeling ontvang wat insluit eerstehulp, noodbehandeling en opvolg-sorg soos benodig. Geen vergoeding, terugbetaling, of gratis mediese sorg word verskaf deur Wayne State Universiteit of die Universiteit van Kaapstad nie. Laat die navorser onmiddelik weet as jy dink dat u kind 'n navorsingsverwante besering opgedoen het.

Studiekostes: Daar sal geen koste wees vir u of u kind om aan hierdie navorsing deel te neem nie, en jy en u kind sal deur ons bestuurder vervoer word na die laboratorium by UK en Tygerberg Hospitaal.

Vergoeding: Vir u deelname aan hierdie navorsingstudie sal ons u R150 (\$25) gee vir elke besoek en 'n foto van u kind, en vir u kind sal ons 'n klein geskenkie gee. Ons sal ook vir u en u kind ontbyt en middagete gee elke keer as julle na UK of Tygerberg Hospitaal toe kom.

Vertroulikheid: Ons sal alle inligting wat ons tydens die studie versamel oor u en u kind geheim hou tot die mate waartoe die wet dit toelaat. Hierdie inligting sal nie gebruik word op enige manier wat enigiemand anders sal toelaat om te weet wat jy of u kind vir ons vertel het nie, behalwe dat tekens van kindermishandeling of –verwaarlosing aan die toepaslike owerhede gerapporteer sal word, soos deur die wet vereis. Jy en u kind sal in ons navorsingsrekords slegs deur 'n kodenommer geïdentifiseer word en julle name sal nie op die rekords verskyn nie. Ons sal nie inligting uitgee wat u of u kind by name noem nie tensy jy ons skriftelik toestemming gee, maar u rekords mag hersien word deur die studie borg, die Menslike Navorsings Komitee by Wayne State Universiteit, of regeringsliggame met toepaslike regulatoriese oorsig. Die lys wat deelnemers se identifikasienommers met hul name verbind sal gestoor word in geslote kabinette in die navorsingslaboratorium. Slegs personelede wat nodig het om u telefonies of persoonlik te kontak sal toegelaat word om na hierdie leërs te kyk. Inligting vanaf hierdie studie, insluitend foto's en videos mag aangebied word by wetenskaplike vergaderings of

joernale of vir opleidingsdoeleindes gebruik word, maar u en u kind se name sal geheim gehou word.

Vrywillige Deelname/Onttrekking: Deelname aan hierdie studie is vrywillig. Jy mag besluit om u kind aan die studie te laat deelneem en later van besluit verander en die studie los. Jy en u kind is ook vry om enige vrae nie te beantwoord nie, of om enige taak te stop voordat dit klaar is. Onttrekking aan die studie sal geen probleme vir u of u kind veroorsaak nie. Die navorser of die borg mag u kind se deelname aan hierdie studie stop sonder dat jy daartoe instem.

Vrae: Indien jy enige vrae het nou of in die toekoms, kan jy Drs. Ernesta Meintjes of Christopher Molteno kontak by 021-406-6291 of Dr. Sandra W. Jacobson by 091-313-993-5454. Indien jy enige vrae of bekommernisse het oor u of u kind se regte as 'n deelnemer aan die navorsing, kan jy die voorsitters kontak van die Universiteit van Kaapstad Navorsings-Etik Komitee (021 406-6338) of die Wayne State Universiteit se Menslike Navorsings Komitees (001-313-577-1628).

Toestemming om aan 'n Navorsingstudie deel te neem: Om vrywilliglik in te stem om u kind te laat deelneem aan hierdie studie, moet jy op die lyn hieronder teken. Indien jy besluit om met u kind deel te neem, mag jy of u kind enige tyd stop. Jy gee nie enige van u of u kind se regte op deur hierdie vorm te teken nie. U handtekening wys dat jy hierdie hele toestemmingsvorm gelees het of dat dit aan u voorgelees is, insluitend die risiko's en voordele, en dat ons al u vrae beantwoord het. Ons sal vir u 'n kopie van hierdie toestemmingsvorm gee om huis toe te neem.

_____	_____
Handtekening van Ouer of Wetlik Gemagtigde Voog	Datum
_____	_____
Naam in drukskrif van Ouer of Wetlik Gemagtigde Voog	Tyd
_____	_____
Mondelinge Instemming (kinders van ouderdom 7-12)	Datum
_____	_____
**Handtekening van Getuie (wanneer van toepassing)	Datum
_____	_____
Naam van Getuie in drukskrif	Tyd
_____	_____
Handtekening van Persoon wat Toestemming neem	Datum
_____	_____
Naam in drukskrif van Persoon wat Toestemming neem	Tyd

**Gebruik wanneer toestemmingsvorm aan ouer voorgelees is (bv. wanneer ongeletterd, wetlik blind, vertaal in 'n vreemde taal).

Appendix D

Informed Assent Form used for Neuroimaging Study (English and Afrikaans versions)

Documentation of Adolescent Assent Form

(ages 13-17)

Title: White Matter Integrity and Executive Functioning in FASD

Why am I here?

This is a research study. Only people who choose to take part are included in research studies. You are being asked to take part in this study because you are one of a large group of children who have been taking part in this study since you were born and have taken part in visits as an infant and at 5 years of age. We are inviting you to take part in the next phase of this study. Please take time to make your decision. Talk to your family about it and be sure to ask questions about anything you don't understand.

Why are they doing this study?

This study is being done to find out how children learn and remember things and solve simple problems. We are trying to understand whether and how diet, alcohol, smoking, and drug exposure during pregnancy may affect development. We study children at different ages using different tasks to see how they grow and develop.

What will happen to me?

Here at University of Cape Town, we will be studying what happens when you feel a puff of air in your eye. You will sit in a chair wearing a special helmet and will watch a video. From time to time, you will feel a puff of air from the helmet and sometimes you will hear a tone. You will also do simple tasks involving tapping your finger, naming pictures, learning lists of words, reading and arithmetic, puzzles, mazes, memory and computer tasks, and tasks about how other people feel and understand another person's point of view. We will also weigh you, measure how tall you are, take a photo, and check how well you can see. You will spend this morning here and will come back to University of Cape Town another day to do the air puff task and the other tasks that I mentioned.

The second part of the study involves neuroimaging, which is a new way to learn about the brain by taking pictures of the brain. These pictures can help us better understand how the brain works. For this part of the study we will drive you and your mother to Tygerberg Hospital. During the neuroimaging, you will lie on a plastic bed that slides into a large machine called a scanner. We will ask you to lie as still as possible while the pictures are

being taken. Taking these pictures of the brain does not hurt and is used everyday by many people in the hospital. During some of the time in the scanner, you will watch videos and during some of the time you will do simple tasks involving tapping your finger or doing simple puzzles, or reading and arithmetic, or learning and memory, or looking at pictures and figuring out if two people seem to have the same feeling. There will be one session in the scanner.

We will also ask you to give us a sample of your spit (saliva) and have a nurse take a small amount of blood from your arm to study how your genes (family characteristics that you get from your parents) affect how you do these tasks and how you act.

How long will I be in the study?

You will be in the study for this phase two days for about 3-4 hours at our laboratory at University of Cape Town (including breakfast, a snack, and lunch) and one visit involving about 45-50 minutes in the scanner and 1 hour of training and assessment outside the scanner at Tygerberg Hospital.

Will the study help me?

You will not benefit from being in this study; however, information from this study may help other people in the future better understand how the brain performs different tasks and whether diet, alcohol, smoking, or drug exposure during pregnancy affects how the brain performs.

While taking part in this phase of the research study, we will give you a small gift and a photo taken of your brain at the end of the scanning. We will provide breakfast, a snack, and lunch each time you come to our laboratory at University of Cape Town or Tygerberg Hospital.

Will anything bad happen to me?

There are no risks from being in the scanner at Tygerberg Hospital or from any of the tasks we do with you in our laboratory at University of Cape Town. The risk of drawing blood include some temporary discomfort swelling and rarely infection. These risks will be small because the blood will be taken by a trained person (nurse/technician). Some people feel nervous in a small closed space, such as when they are in the scanner. You will practice what it is like in a pretend scanner beforehand. We will give you earplugs or headphones so that the loud banging of the scanner will not bother you. There is a button you can press to ask

questions or stop the scan at anytime. You can see out of the scanner at all times, and we will not start until you are comfortable with the set-up.

Do my parents or guardians know about this? (If applicable)

This study information has been given to your parents/guardian and they said that you could take part in the study. You can talk this over with them before you decide.

Research Related Injuries

In the event that this research related activity results in an injury, treatment will be made available including first aid, emergency treatment, and follow-up care as needed. Care for such will be billed in the ordinary manner to you or your insurance company/South African public assistance. No reimbursement, compensation, or free medical care is offered by Wayne State University or the University of Cape Town. If you think that you have suffered a research related injury, please contact the Cape Town PI (Dr. Christopher Molteno) right away at 021-406-6291.

What about confidentiality?

Every reasonable effort will be made to keep your records (medical or other) and/or your information confidential, however we do have to let some people look at your study records.

We will keep your records private unless we are required by law to share any information.

The law says we have to tell someone if you might hurt yourself or someone else. The study doctor can use the study results as long as you cannot be identified.

The following information must be released/reported to the appropriate authorities if at any time during the study there is concern that:

- child abuse or elder abuse has possibly occurred,
- you disclose illegal criminal activities, illegal substance abuse or violence

What if I have any questions?

For questions about the study please call Dr. Christopher Molteno at 021-406-6291. If you have questions or concerns about your rights as a research participant, the Chair of the Institutional Review Board can be contacted at 001-313-577-1628 or you can contact the Chair of the University of Cape Town Research Ethics Committee at 021-406-6338.

Do I have to be in the study?

You don't have to be in this study if you don't want to or you can stop being in the study at any time. Please discuss your decision with your parents and researcher. No one will be angry if you decide to stop being in the study.

AGREEMENT TO BE IN THE STUDY

Your signature below means that you have read the above information about the study and have had a chance to ask questions to help you understand what you will do in this study. Your signature also means that you have been told that you can change your mind later and withdraw if you want to. By signing this assent form you are not giving up any of your legal rights. You will be given a copy of this form.

Signature of Participant (13 yrs & older)

Date

Printed name of Participant (13 yrs & older)

**Signature of Witness (When applicable)

Date

Printed Name of Witness

Signature of Person who explained this form

Date

Printed Name of Person who explained form

** Use when participant has had consent form read to them (i.e., illiterate, legally blind, translated into foreign language).

Afrikaans Informed Assent Form used for Neuroimaging Study

Dokumentasie van Adollesente Instemming Form

(Ouderdomme 13-17)

Titel: Witstof Integriteit en Uitvoerende Funksionering in FASD

Hoekom is ek hier?

Hierdie is 'n navorsingstudie. Slegs mense wat kies om deel te neem word ingesluit by navorsingstudies. Jy word gevra om deel te neem aan hierdie studie omdat jy een van 'n groot groep kinders is wat al aan hierdie studie deelneem vandat jy gebore is en het deel geneem aan besoeke toe jy 'n baba was en toe jy 5 jaar oud was. Ons nooi jou uit om deel te neem aan die volgende fase van hierdie studie. Vat asseblief jou tyd om 'n besluit te neem. Gesels met jou familie daaroor en maak seker om vrae te vra oor enige iets wat jy nie verstaan nie.

Hoekom doen hulle hierdie studie?

Hierdie studie word gedoen om uit te vind hoe kinders dinge leer en onthou en hoe hulle eenvoudige probleme oplos. Ons probeer om te verstaan hoe en of dieet, alkohol, rook, en blootstelling aan dwelms gedurende swangerskap ontwikkeling kan beïnvloed. Ons bestudeer kinders op verskillende ouderdomme met verskillende take om te sien hoe hulle groei en ontwikkel.

Wat sal met my gebeur?

Hier by die Universiteit van Kaapstad, sal ons bestudeer wat gebeur wanneer jy 'n blasie lug in jou oog voel. Jy sal in 'n stoel sit met 'n spesiale helm op jou kop en jy sal 'n video kyk. Elke nou en dan, sal jy 'n lugblasie uit die helm voel kom en soms sal jy 'n geluid hoor. Jy sal ook eenvoudige take doen waartydens jy jou vinger moet tik, prentjies benoem, lyste met woorde leer, lees en somme doen, legkaarte doen, doolhowe doen, geheue en rekenaar take doen en take oor hoe ander mense voel en 'n ander persoon se oogpunt insien. Ons sal jou ook weeg, meet hoe lank jy is, 'n foto neem en kyk hoe goed jy kan sien. Jy sal vanoggend hier spandeer en sal terug kom na die Universiteit van Kaapstad toe op 'n ander dag om die lugblasie taak en die ander take wat ek genoem het te doen.

Die tweede deel van die studie behels neurobeelding, wat 'n nuwe manier is om van die brein te leer deur prentjies te neem van die brein. Hierdie prentjies kan ons help om beter te verstaan hoe die brein werk. Vir hierdie deel van die studie sal ons jou en jou ma na Tygerberg Hospitaal toe vervoer. Gedurende die neurobeelding, sal jy op 'n plastiek bed lê

wat in 'n groot masjien inskuif wat 'n skandeerder genoem word. Ons sal jou vra om so stil as moontlik te lê terwyl die prentjies geneem word. Die afneem van hierdie prentjies (foto's) van die brein maak nie seer nie en word elke dag deur baie mense in die hospitaal gebruik. Vir 'n gedeelte van die tyd in die skandeerder sal jy na videos kyk, en vir 'n gedeelte van die tyd sal jy eenvoudige take doen waartydens jy jou vinger moet tik of eenvoudige legkaarte doen, of lees en somme doen, of dinge probeer onthou, of na prentjies kyk en probeer uitwerk of twee mense dieselfde gevoelens voel. Daar sal een sessie in die skandeerder wees. Ons sal jou ook vra om vir ons 'n bietjie van jou spoeg (speeksel) te gee en 'n verpleegster sal 'n klein hoeveelheid bloed van jou arm neem om te bestudeer hoe jou gene (familie eienskappe wat jy van jou ouers af kry) beïnvloed hoe jy hierdie take doen en hoe jy optree.

Hoe lank sal ek in die studie wees?

Jy sal twee dae in die studie wees vir hierdie fase, vir ongeveer 3-4 ure by ons laboratorium by die Universiteit van Kaapstad (insluitend ontbyt, 'n peuselhappie, en middagete) en een besoek van sowat 45-50 minute in die skandeerder en 1 uur van opleiding en assessering buite die skandeerder by Tygerberg Hospitaal.

Sal die studie my help?

Jy sal nie daarby baat om in hierdie studie te wees nie, maar inligting uit hierdie studie kan ander mense in die toekoms help om beter te verstaan hoe die brein verskillende take verrig en of dieet, alkohol, rook, of blootstelling aan dwelms gedurende swangerskap beïnvloed hoe die brein werk.

Terwyl jy in hierdie fase van die navorsing deel neem, sal ons vir jou 'n klein geskenkie gee en 'n foto wat van jou brein geneem is aan die einde van die skandering. Ons sal ontbyt, 'n peuselhappie, en middagete voorsien elke keer as jy na ons laboratorium toe kom by die Universiteit van Kaapstad of Tygerberg Hospitaal.

Sal enige iets sleg met my gebeur?

Daar is geen risiko's verbonde aan om in die skandeerder by Tygerberg Hospitaal te wees nie, of enige van die take wat ons met jou doen in ons laboratorium aan die Universiteit van Kaapstad nie. Die risiko van bloed trek sluit in 'n bietjie tydelike ongemak, swelling en selde infeksie. Hierdie risiko's sal klein wees, want die bloed sal geneem word deur 'n opgeleide persoon (verpleegster/tegnikus).

Sommige mense voel senuweeagtig in 'n klein beperkte spasie, soos wanneer hulle in die skandeerder is. Jy sal voor die tyd oefen hoe dit gaan voel in 'n oefen skandeerder. Ons sal vir jou oorpluisies of oorfone gee sodat die harde geraas van die skandeerder jou nie pla nie.

Daar is 'n knoppie wat jy kan druk om vrae te vra of die skandering te stop op enige tyd. Jy kan te alle tye by die skandeerder uitsien, en ons sal nie begin voordat jy gemaklik is nie.

Weet my ouers of voogde hiervan? (Indien van toepassing)

Hierdie studie inligting is aan jou ouers/voogde gegee en hulle het gesê dat jy kan deel neem aan die studie. Jy kan met hulle hieroor praat voordat jy besluit.

Navorsingsverwante Beserings

Indien hierdie navorsingsverwante aktiwiteite lei tot 'n besering, sal behandeling beskikbaar gemaak word, insluitend eerstehulp, noodbehandeling, en opvolg-sorg soos benodig. Sulke sorg sal betaalbaar wees in die gewone manier deur jou of jou versekerings maatskappy/Suid-Afrikaanse openbare hulp. Geen terugbetaling, vergoeding, of gratis mediese sorg word verskaf deur Wayne State Universiteit of die Universiteit van Kaapstad nie. As jy dink dat jy 'n navorsingsverwante besering opgedoen het, kontak asseblief dadelik die Kaapstad hoofnavorser (Dr Christopher Molteno) by 021-406-6291.

Wat van vertroulikheid?

Elke redelike poging sal aangewend word om jou rekords (mediese of ander) en/of jou inligting konfidensieel te hou, maar ons moet sommige mense na jou studie rekords laat kyk. Ons sal jou rekords geheim hou tensy ons deur die wet vereis word om enige inligting te deel. Die wet sê dat ons iemand moet vertel as jy dalk jouself of iemand anders mag seer maak. Die studie dokter kan die studie resultate gebruik so lank as wat jy nie geïdentifiseer kan word nie.

Die volgende inligting moet vrygelaat word/gerapporteer word aan die toepaslike owerhede indien daar te eniger tyd gedurende die studie kommer is dat:

- kindermisbruik of mishandeling van bejaardes moontlik plaasgevind het,
- jy onwettige kriminele aktiwiteite openbaar, onwettige drank-en dwelmmisbruik, of geweld

Wat as ek enige vrae het?

Vir vrae oor die studie kontak asseblief vir Dr Christopher Molteno by 021-406-6291. Indien jy enige vrae of bekommernisse het oor jou regte as 'n deelnemer aan die navorsing, kan die voorsitter van die Wayne State Universiteit se Menslike Navorsings Komitee gekontak word by 001-313-577-1628 of jy kan die voorsitter van die Universiteit van Kaapstad Navorsings-Etik Komitee kontak by 021-406-6338.

Moet ek in die studie wees?

Jy hoef nie in hierdie studie te wees as jy nie wil nie of jy kan ophou om in die studie te wees op enige stadium. Bespreek asseblief jou besluit met jou ouers en navorser. Niemand sal kwaad wees as jy besluit om op te hou om in die studie te wees nie.

INSTEMMING OM IN DIE STUDIE TE WEES

Jou handtekening hieronder beteken dat jy die bogenoemde inligting oor die studie gelees het, en dat jy kans gekry het om vrae te vra om jou te help verstaan wat jy in hierdie studie gaan doen. Jou handtekening beteken ook dat daar aan jou verduidelik is dat jy later van besluit mag verander en onttrek as jy wil. Jy gee nie enige van jou regte op deur hierdie vorm te teken nie. Ons sal vir jou 'n kopie van hierdie toestemmingsvorm gee.

Handtekening van Deelnemer (13 j. & ouer)

Datum

Naam van Deelnemer in drukskrif (13 j. & ouer)

**Handtekening van Getuie (Wanneer van toepassing)

Datum

Naam van Getuie in drukskrif

Handtekening van Persoon wat vorm verduidelik

het

Datum

Naam van Persoon wat vorm verduidelik het

**Gebruik wanneer toestemmingsvorm aan deelnemer voorgelees is (bv. wanneer ongeletterd, wetlik blind, vertaal in 'n vreemde taal).